

Construction of Pbs.PGK.PCR1

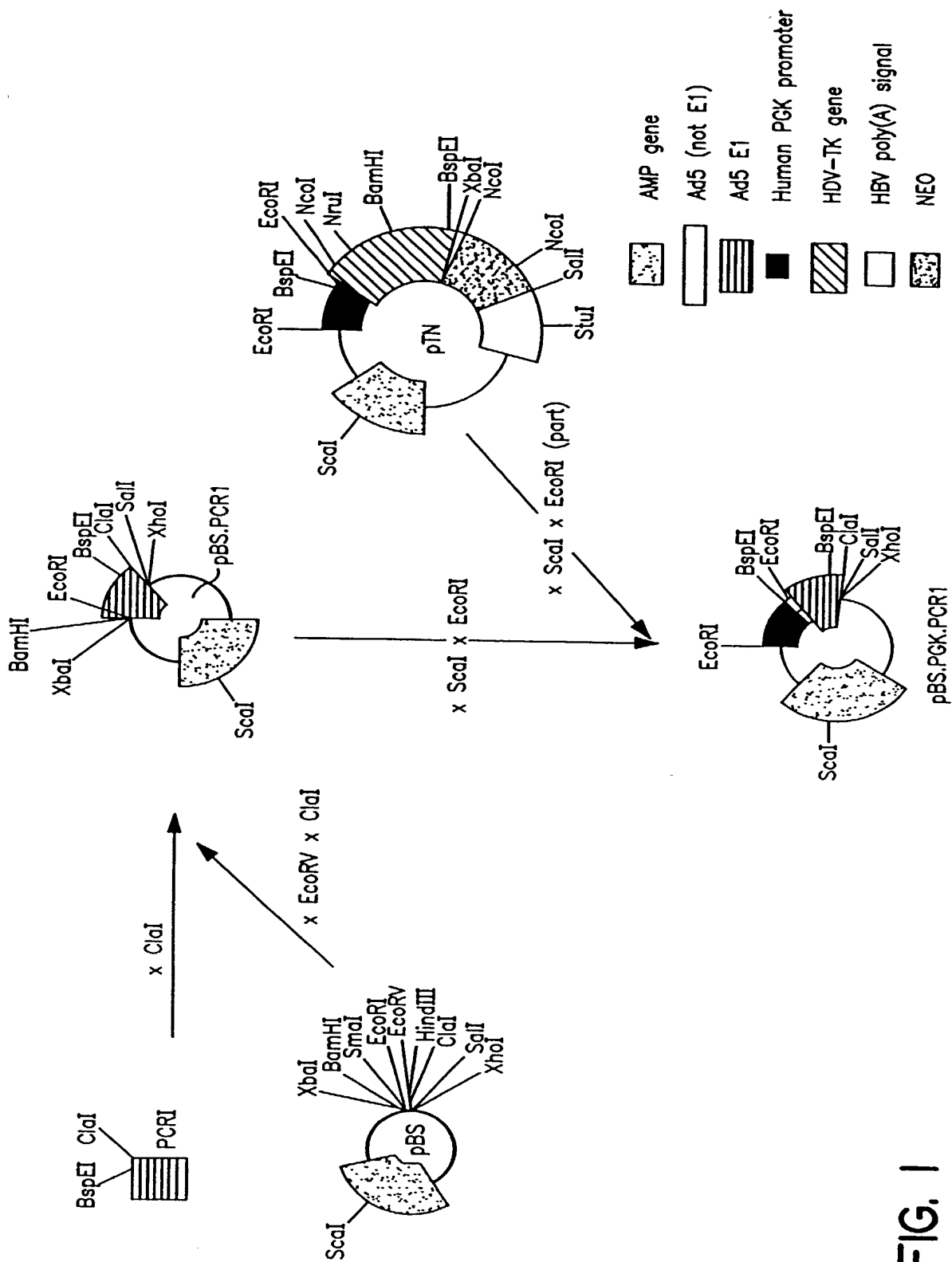


FIG. 1

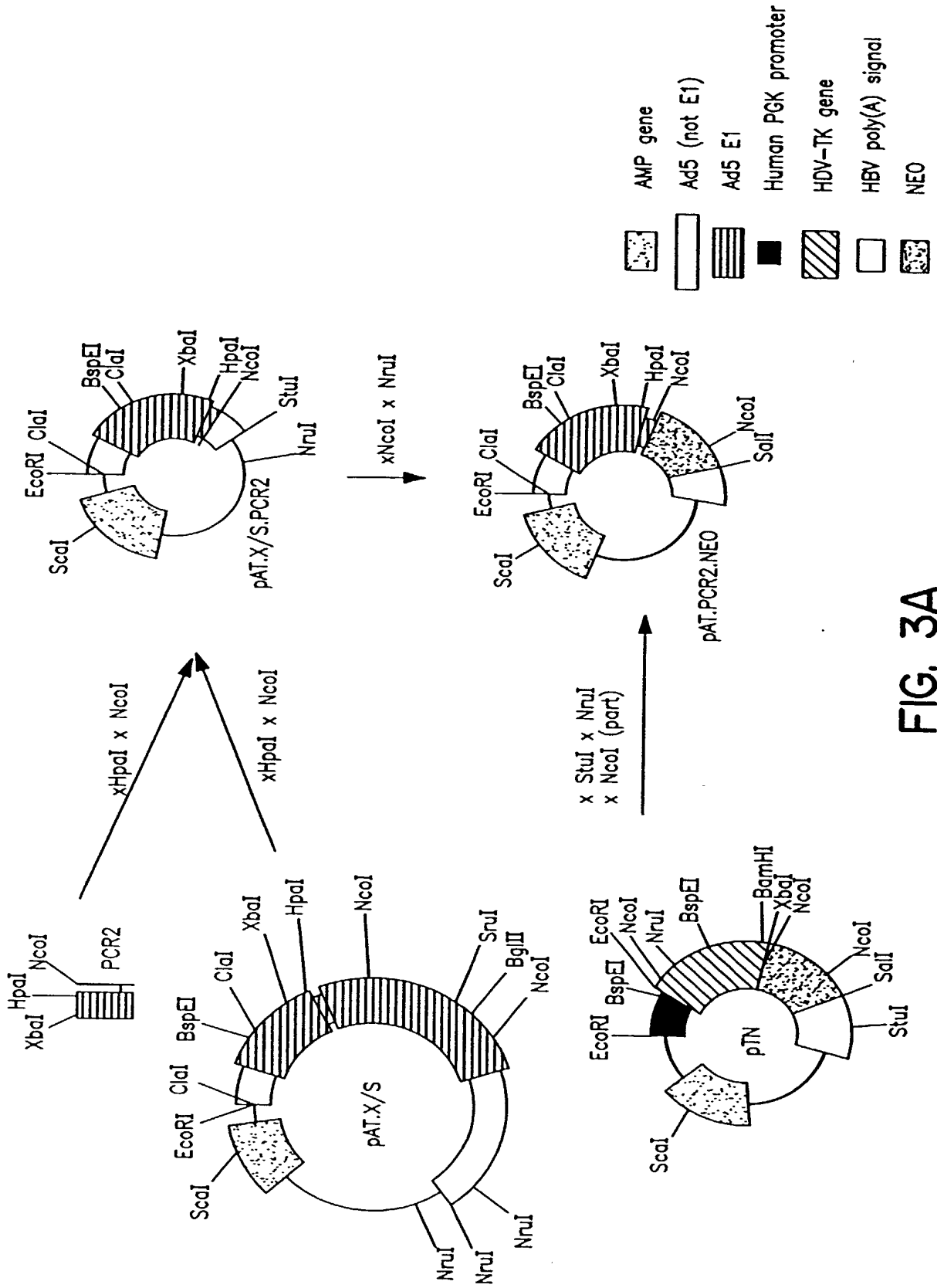


FIG. 3A

Construction of pIG.E1a.NEO

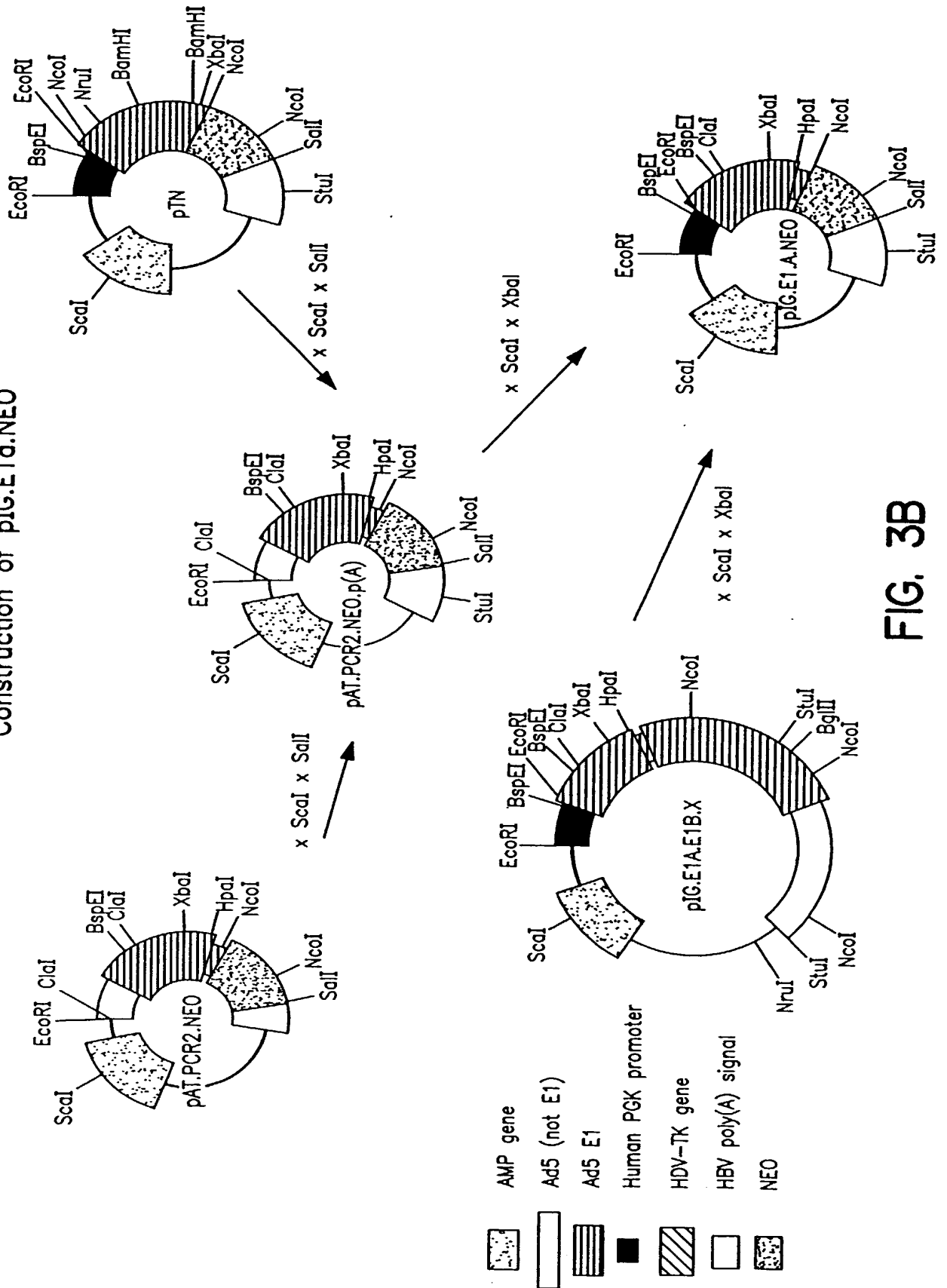
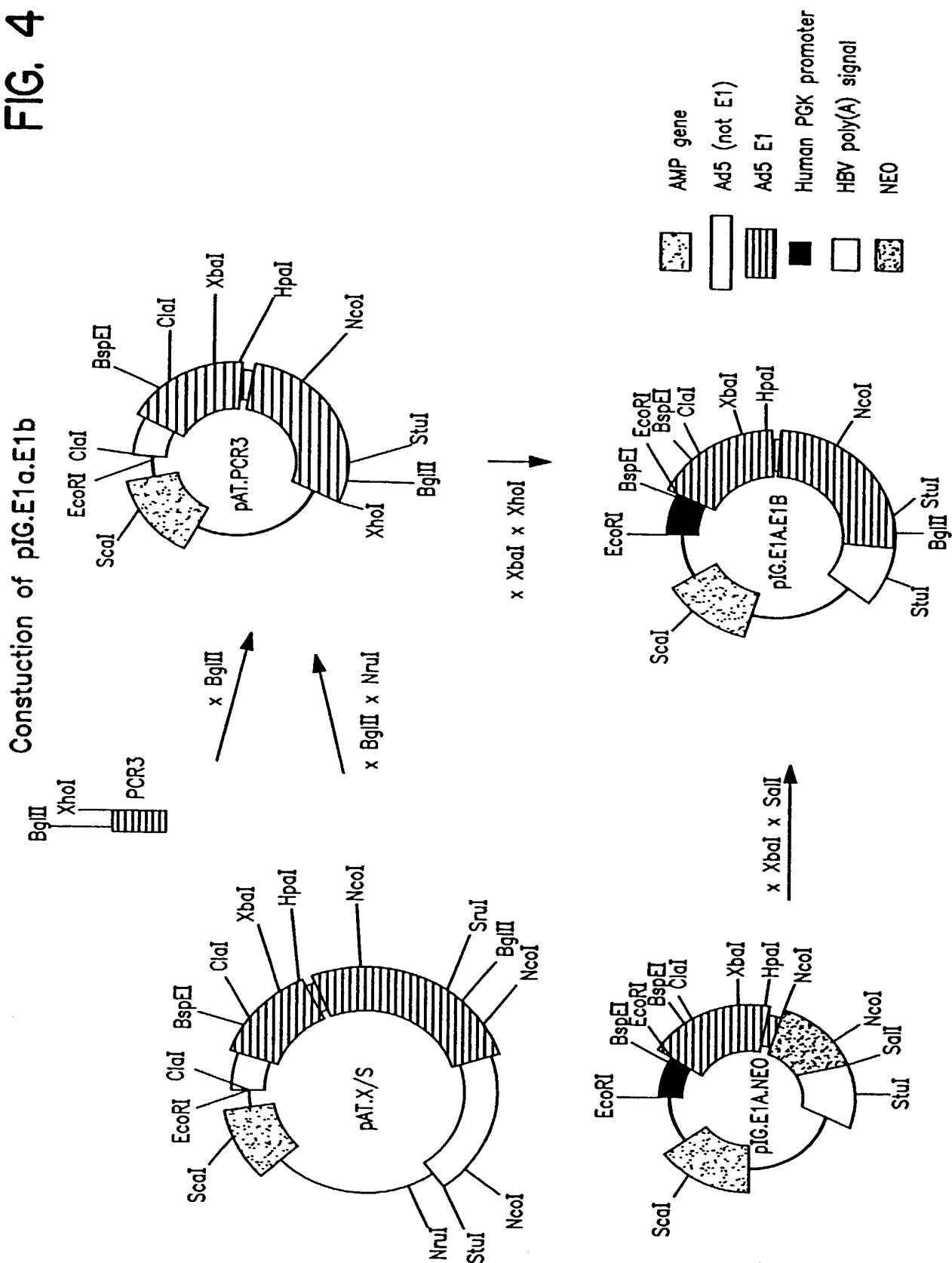


FIG. 3B



Construction of pIG.NEO

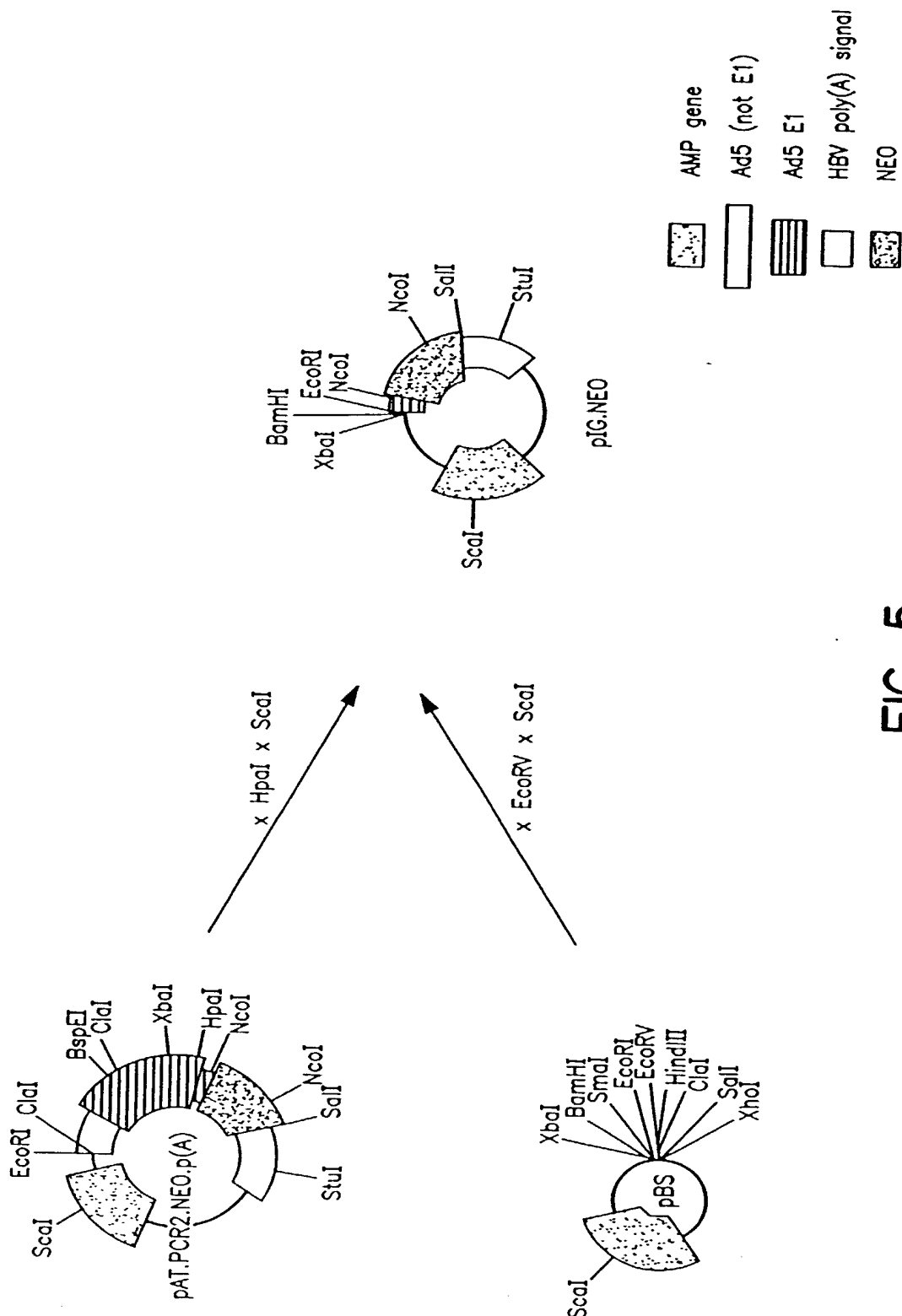
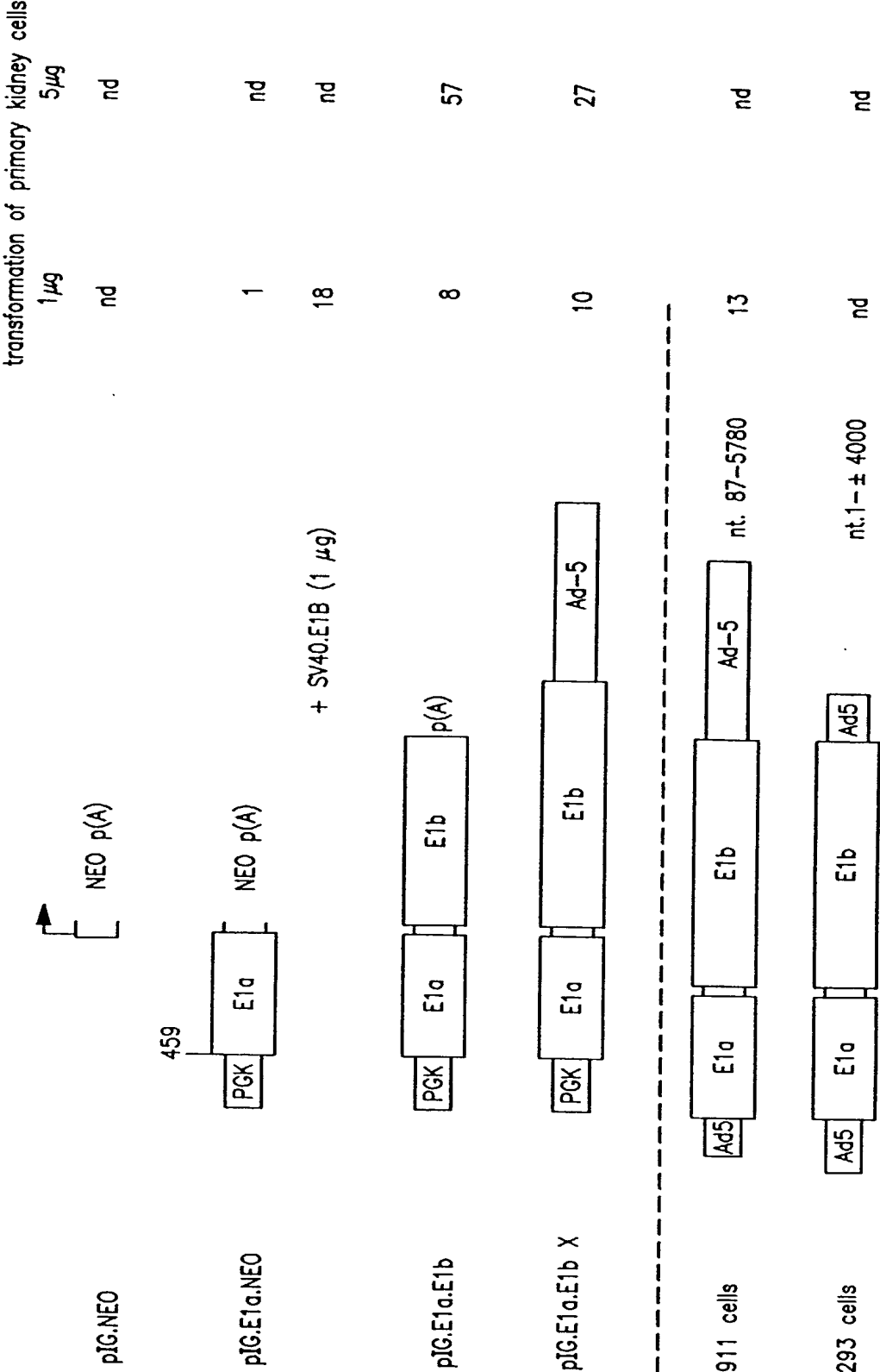


FIG. 5

Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells



*average of 5 plates 21 days after transection

FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones (HER cells transfected with pIG.E1A.E1B)

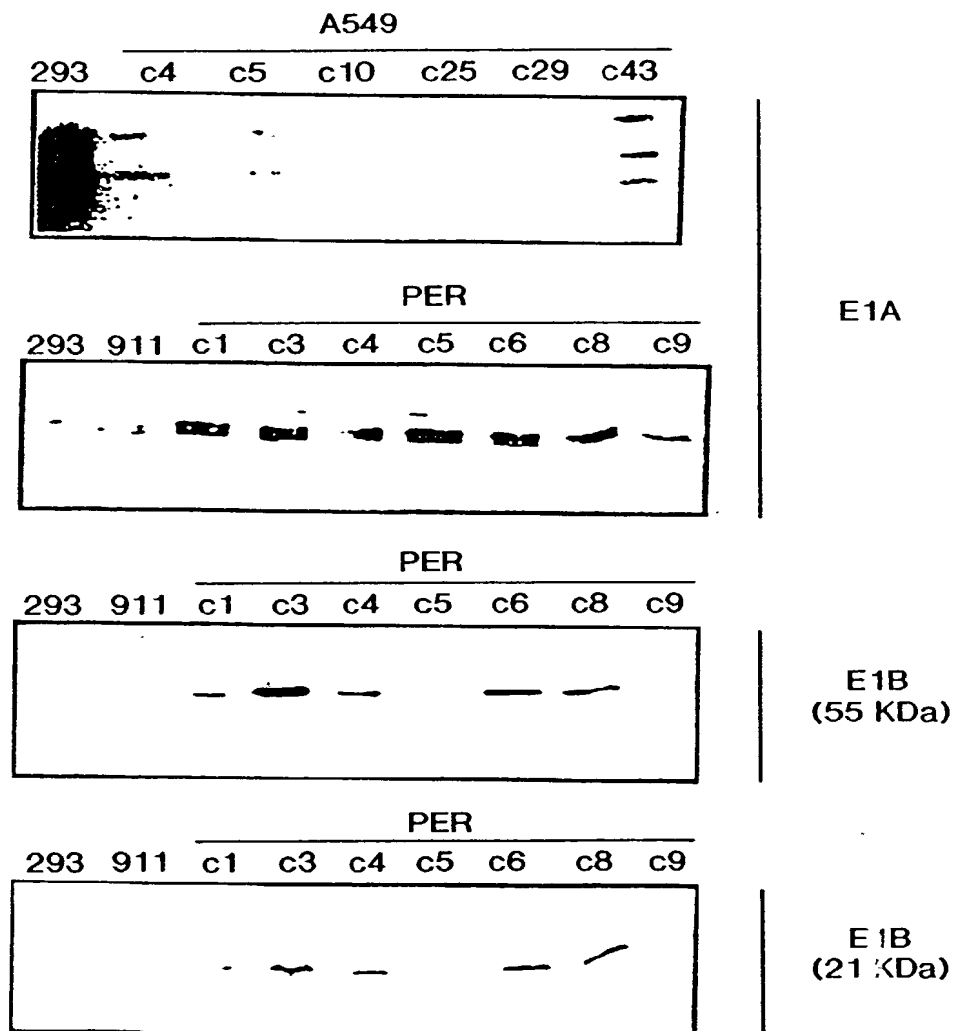


FIG. 7

Southern blot analyses of 293, 911 and PER cell lines

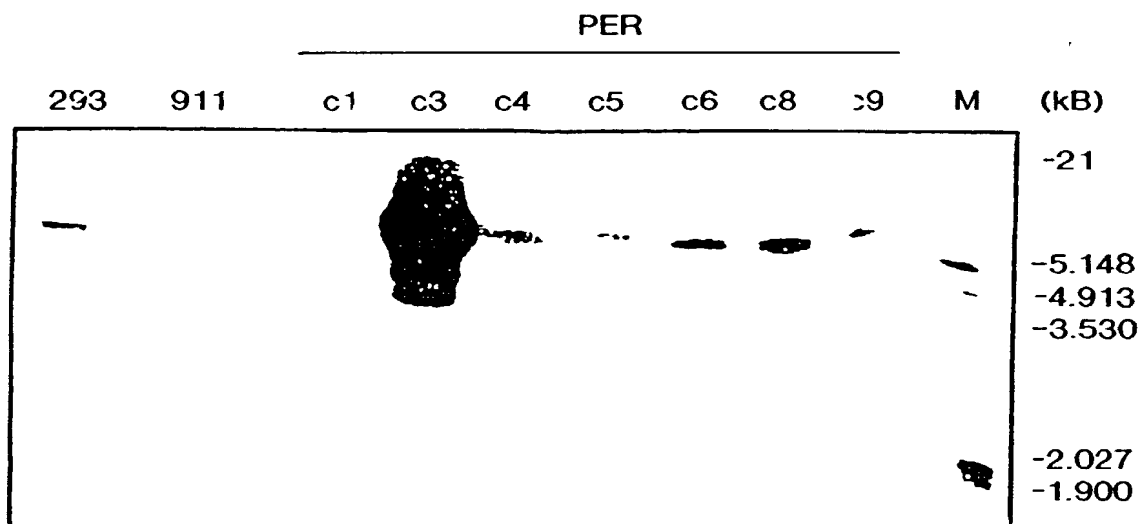


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5 μ g pRSV.lacZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

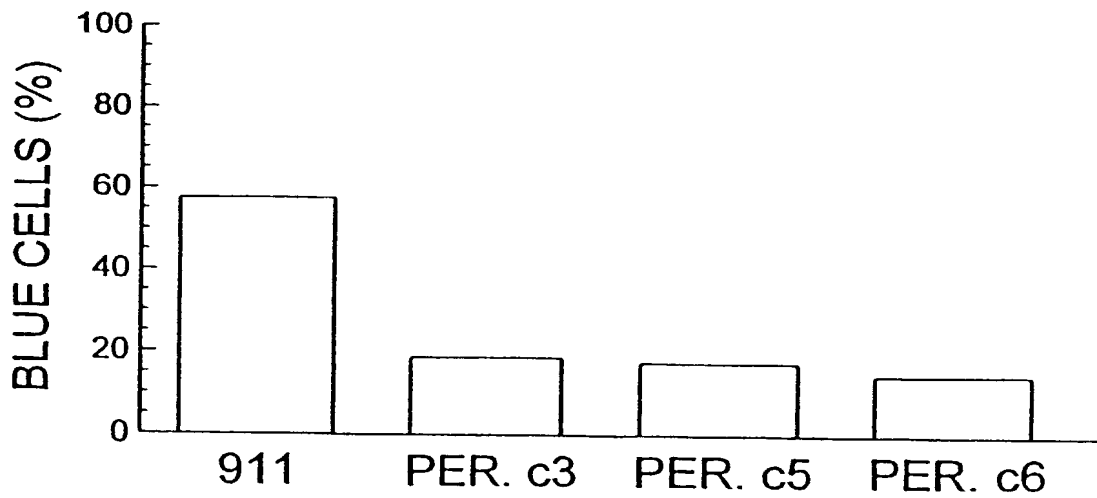


FIG. 9

Construction of pMLP1.TK

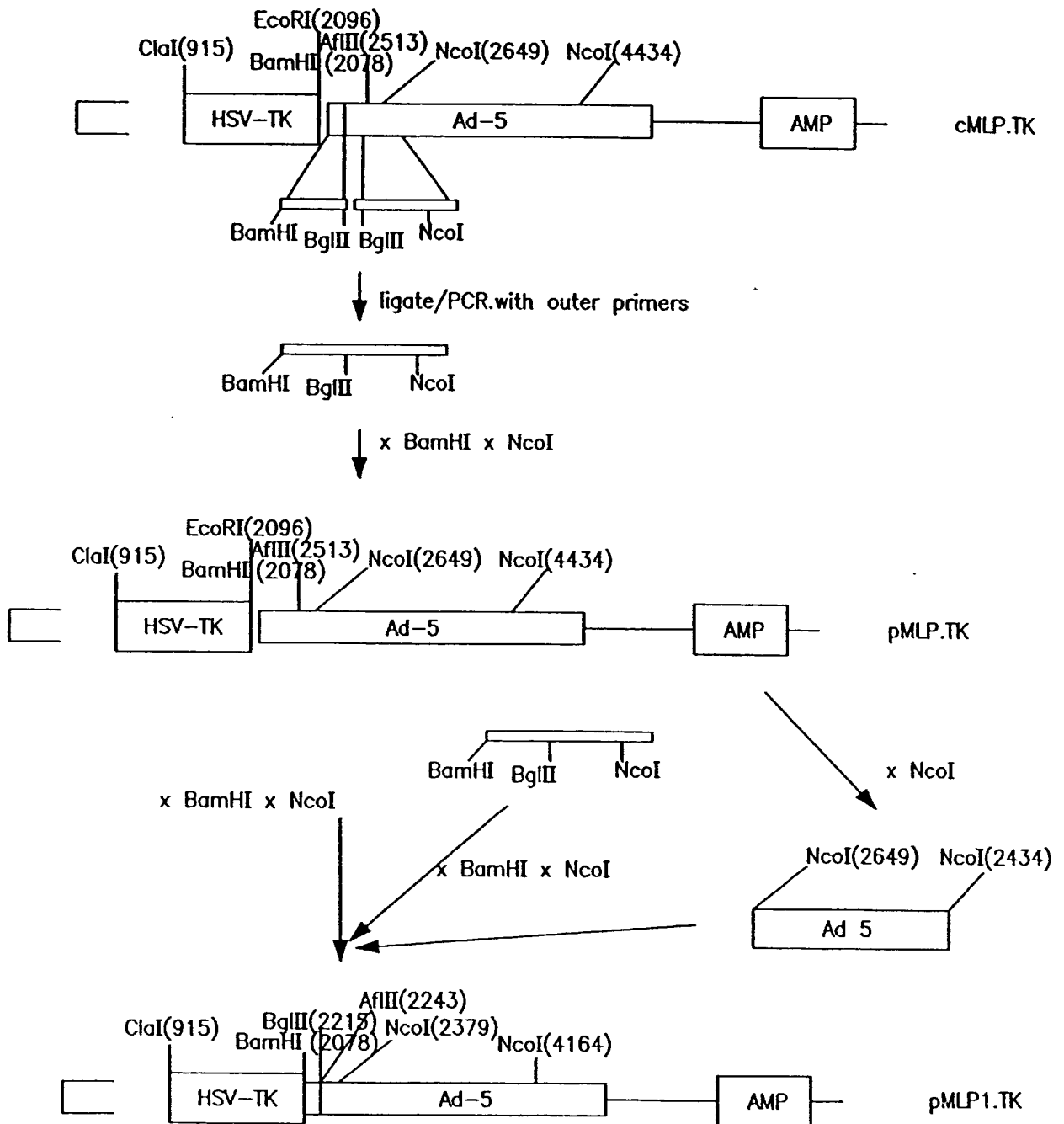


FIG. 10

New recombinant adenoviruses and packaging constructs without sequence overlap

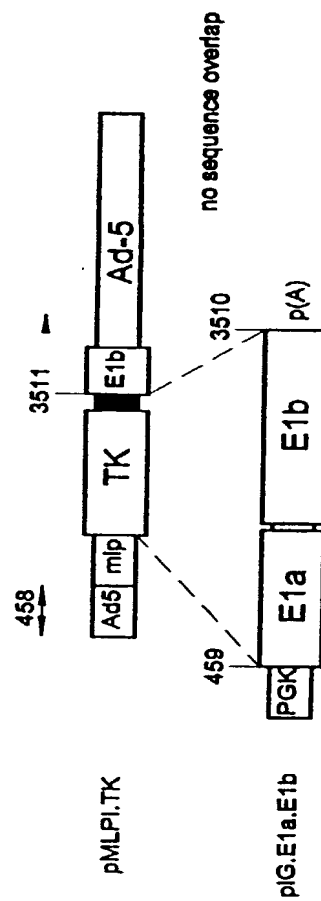
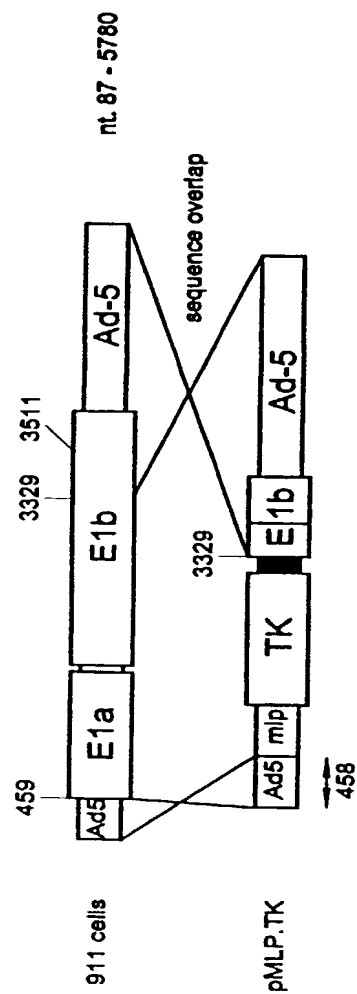
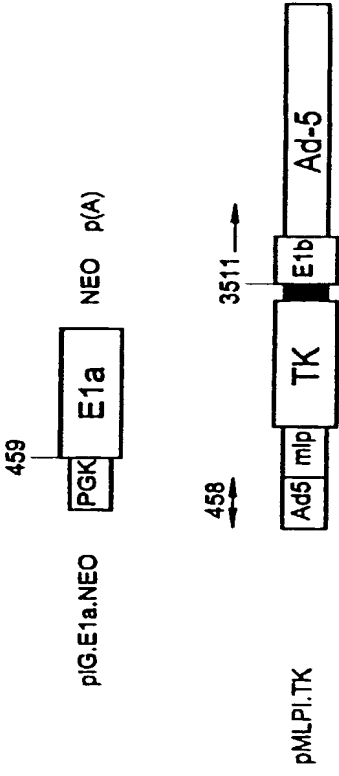


FIG. 11A

Packaging system based on primary cells

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418 **FIG. 1B**

Generation of recombinant adenovirus

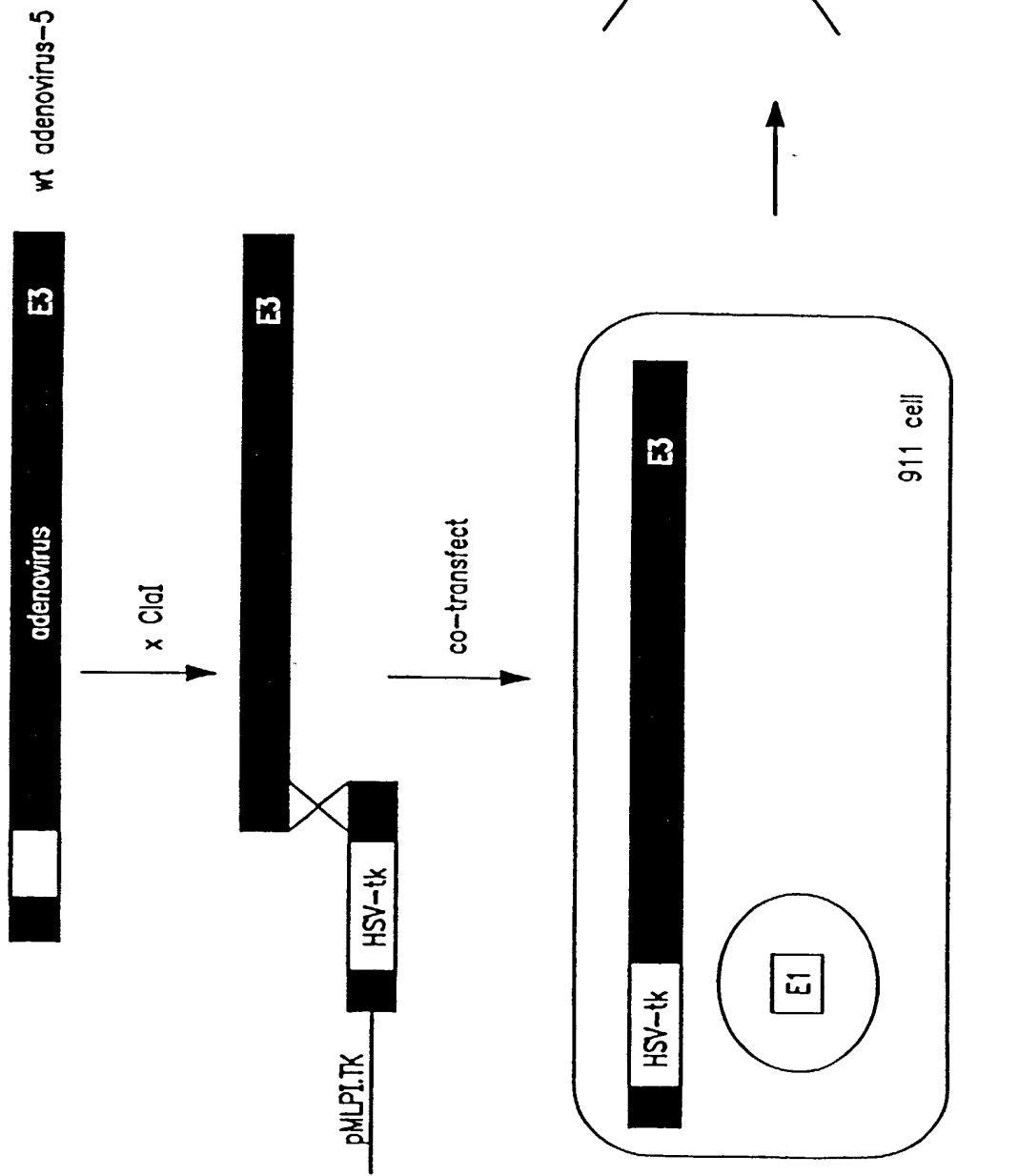


FIG. 12

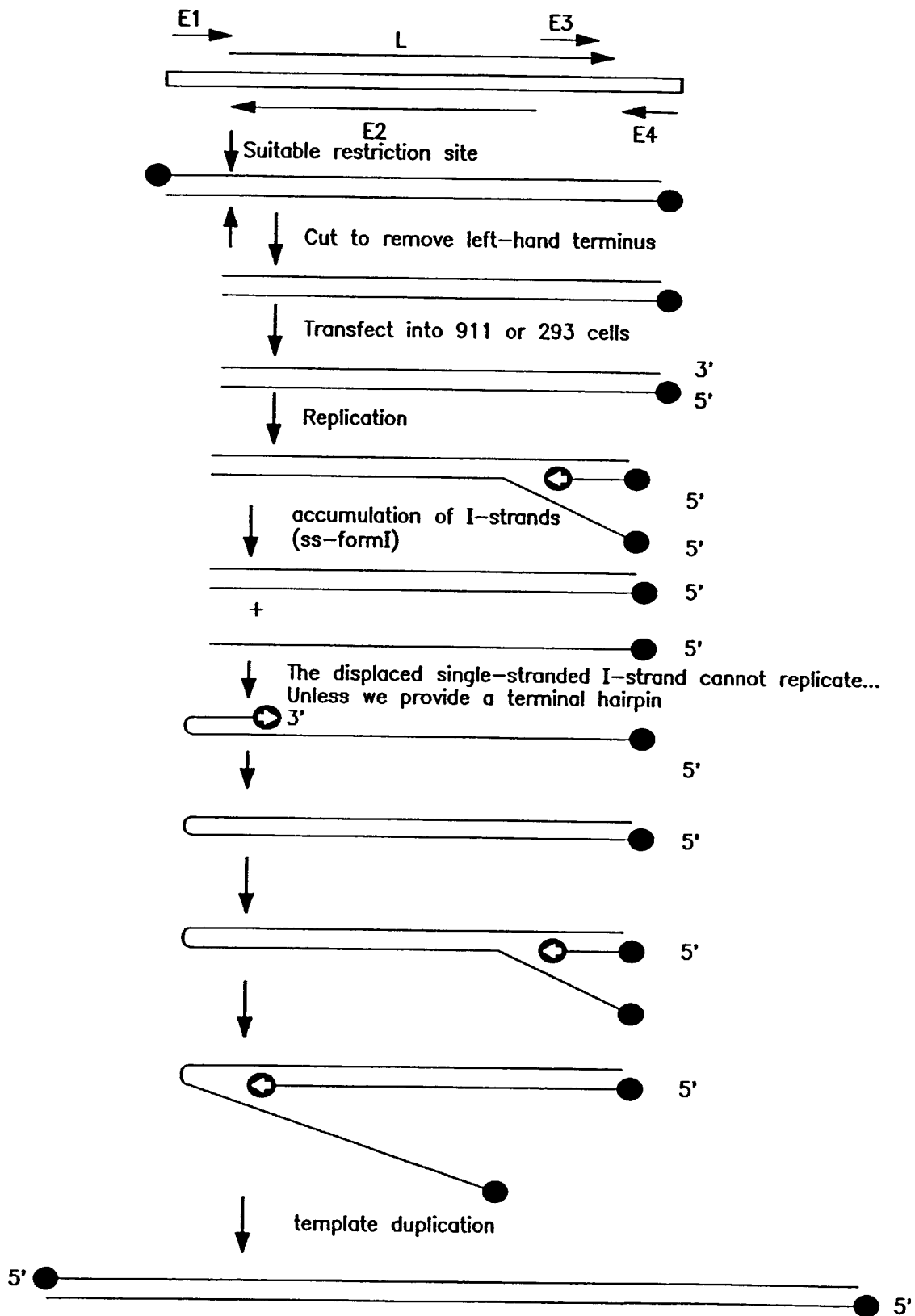


FIG. 13

Replication of Adenovirus

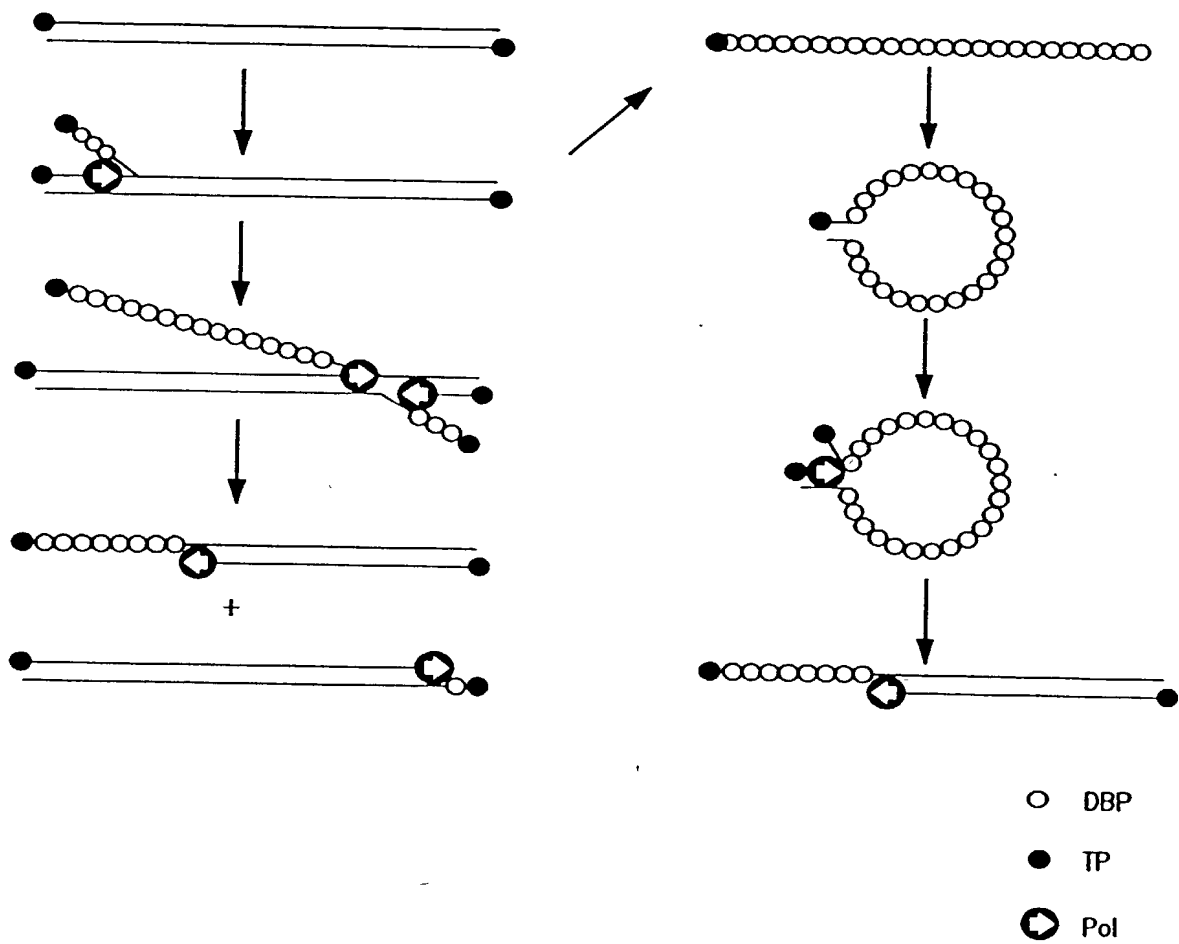


FIG. 14

The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasmid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

```

5'-GTAACTGACCTAGTGCCGCCCCGGGCA
      |||||
3'-GATCACGGCGGGCCCCGA
  
```

FIG. 15

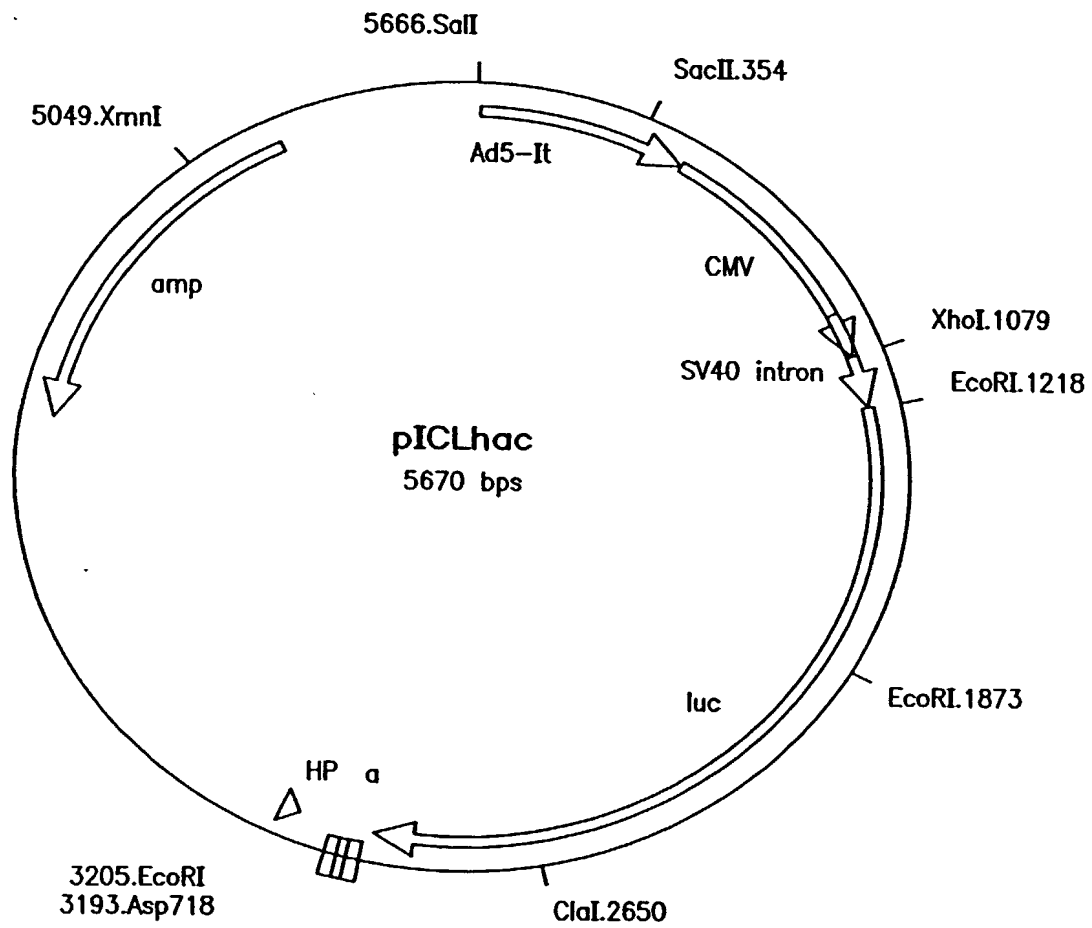


FIG. 16

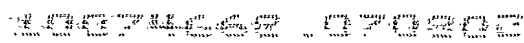


FIG. 17

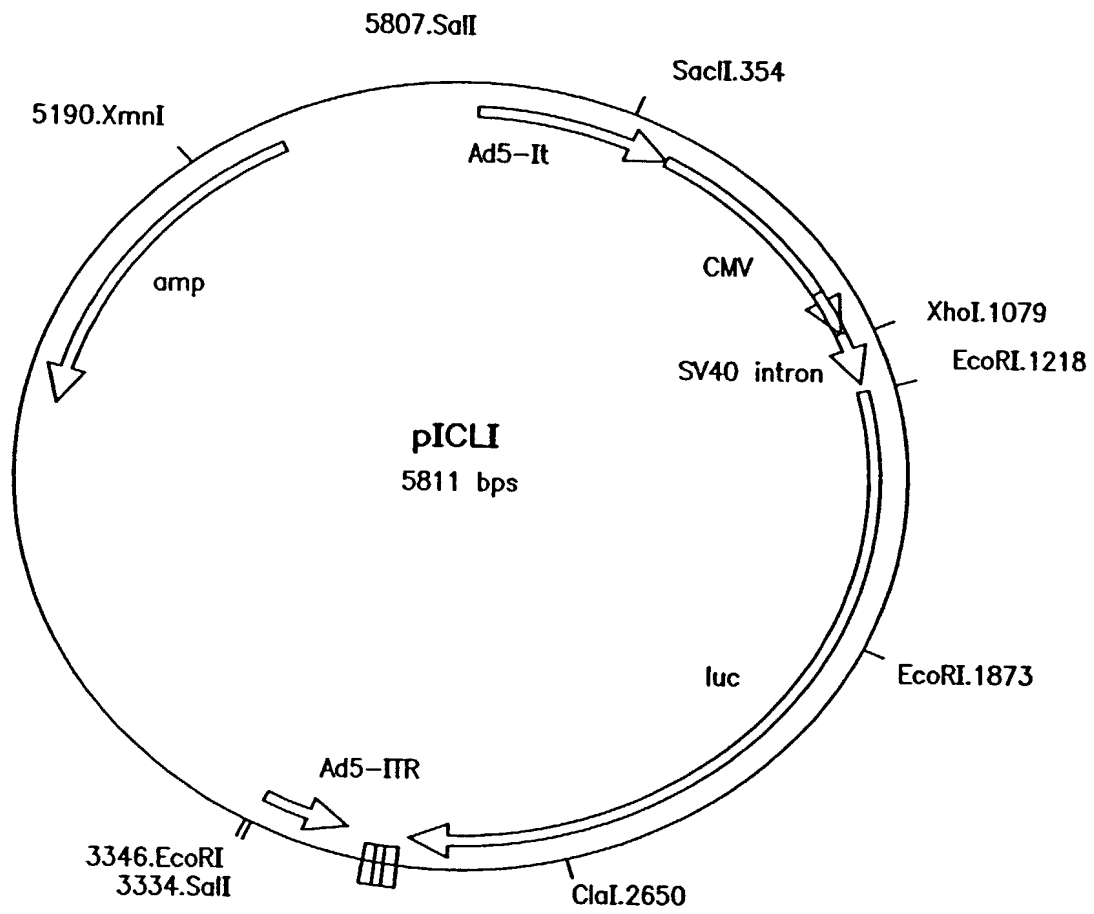


FIG. 18

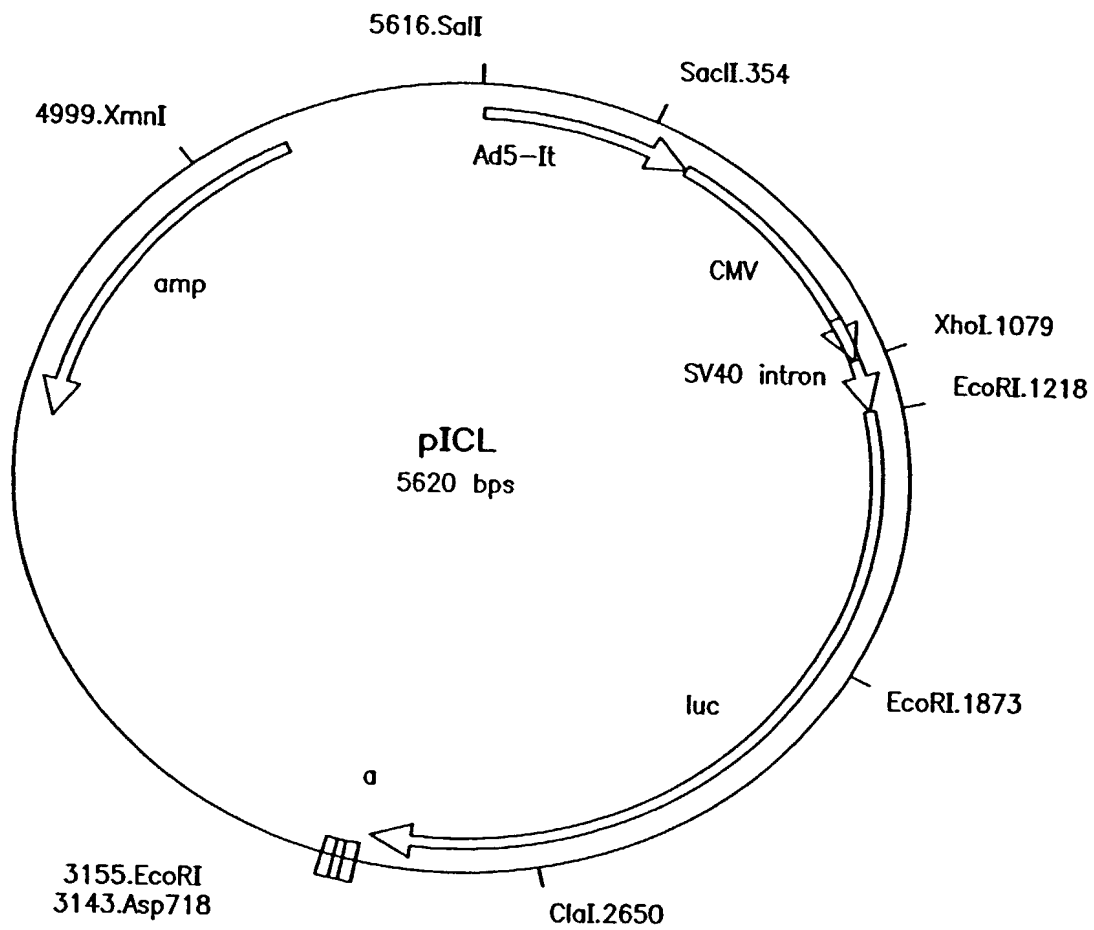


FIG. 19

Cloned adenovirous fragments

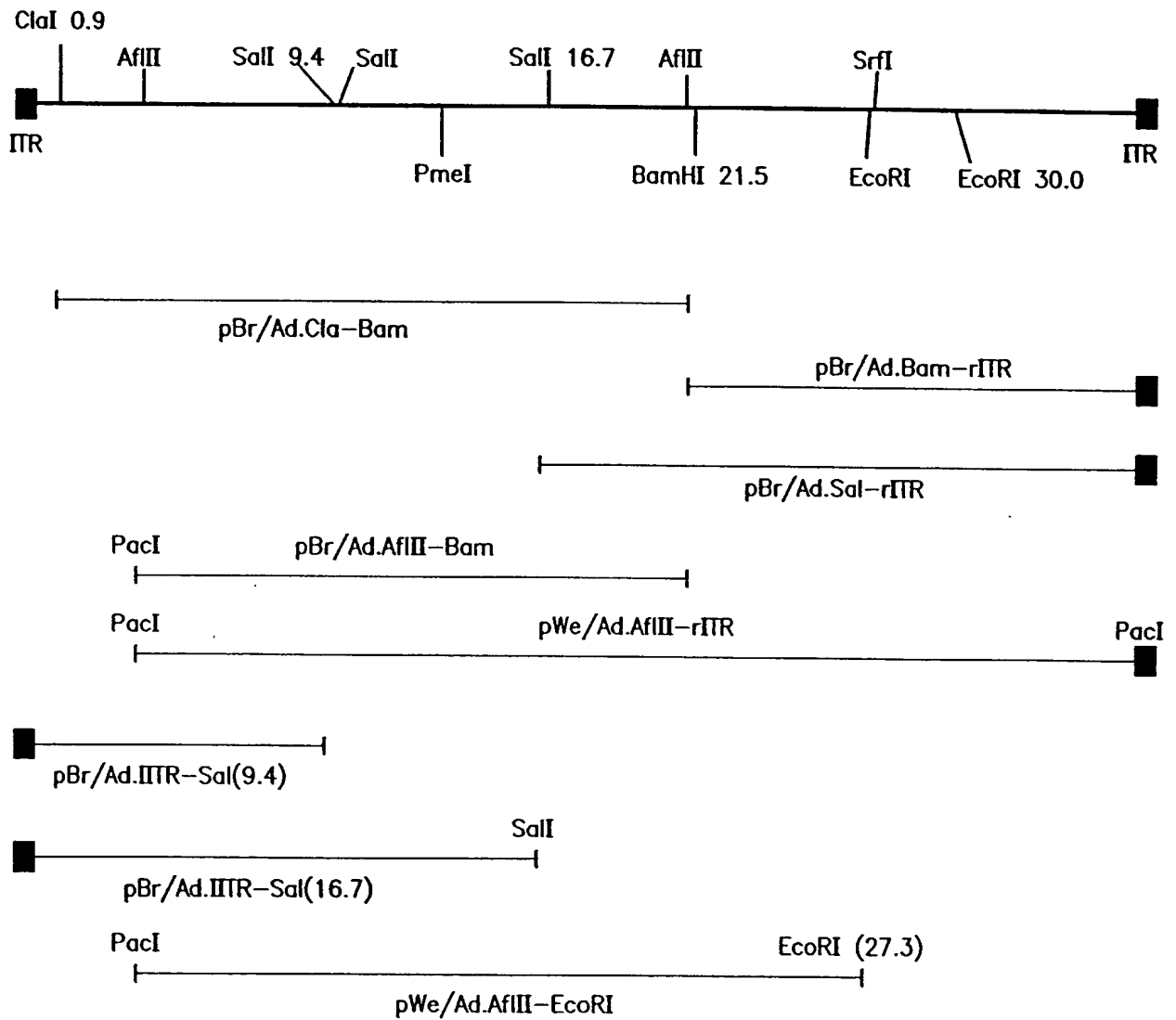


FIG. 20

Adapter plasmid pAd5/L420-HSA

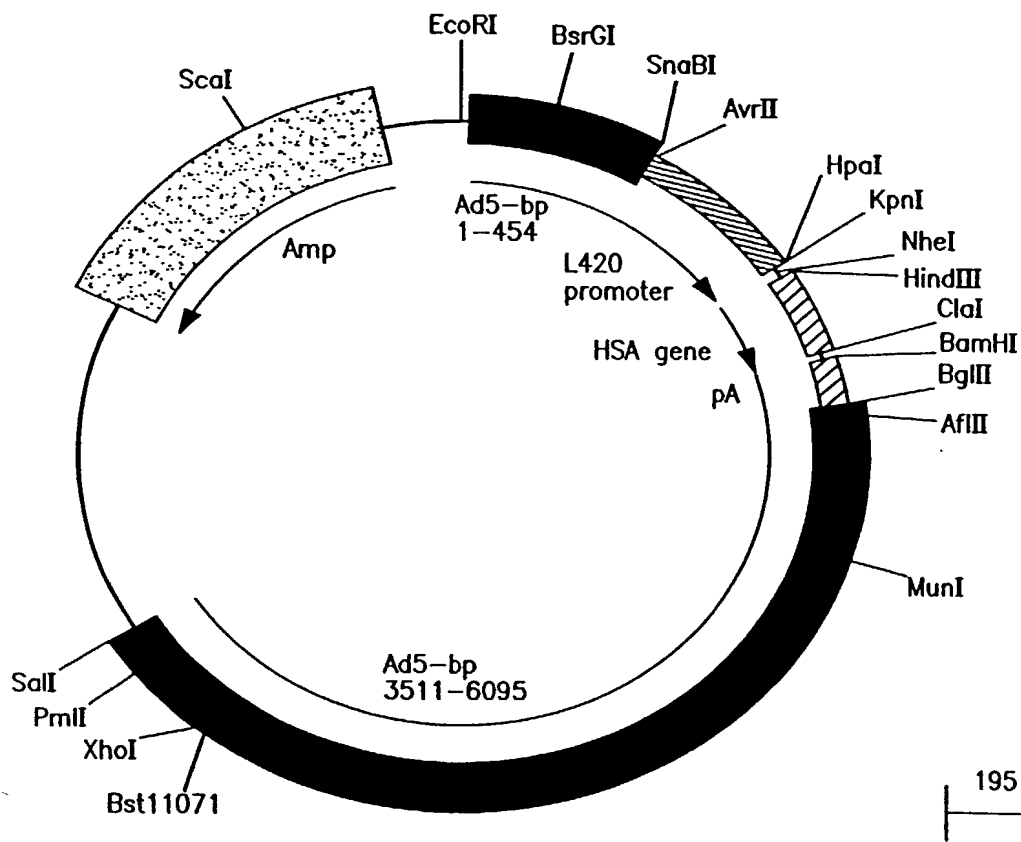


FIG. 21

[illegible]

FIG. 22

Generation of recombinant adenoviruses

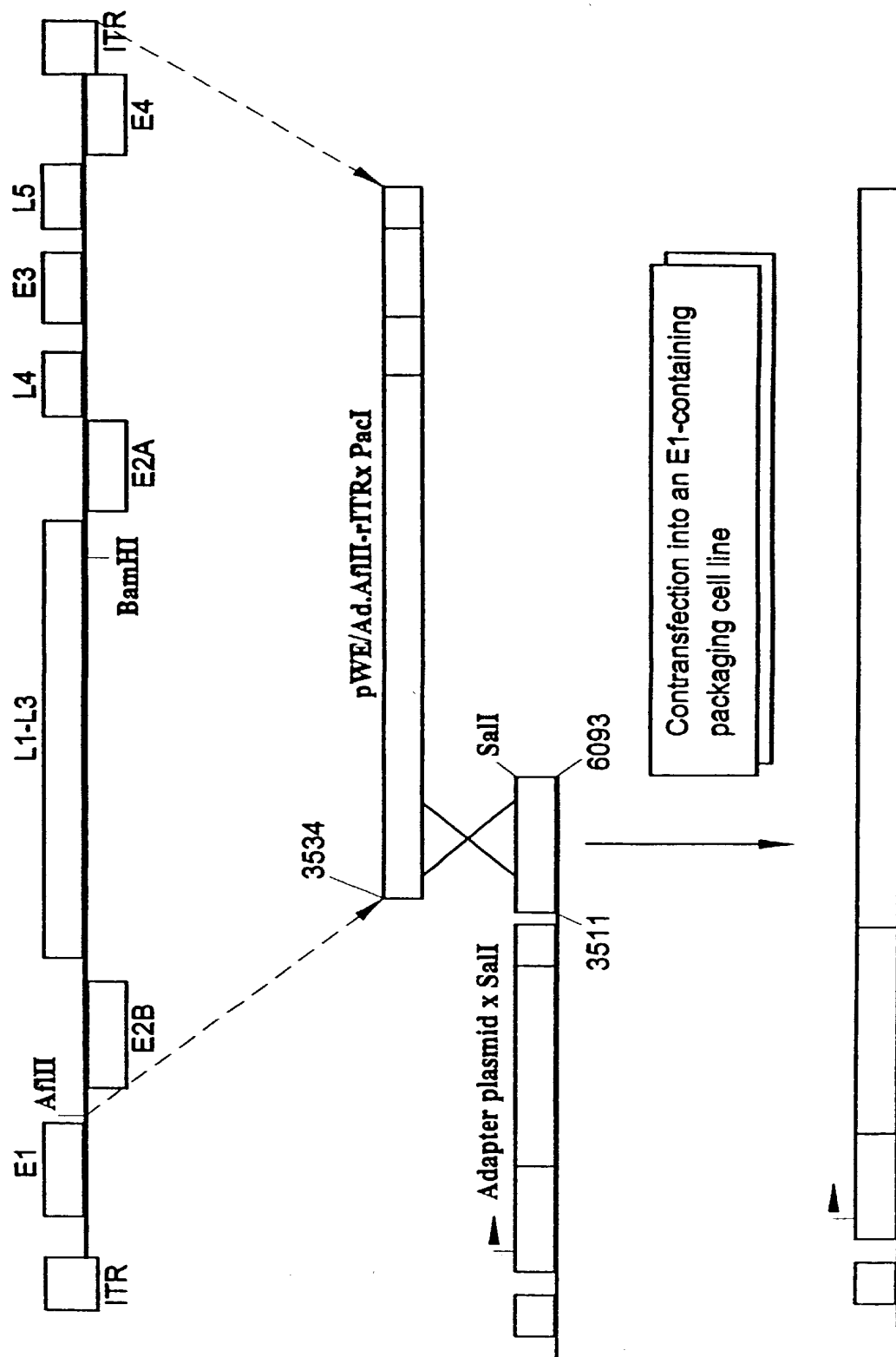


FIG. 23

Minimal adenovirus vector pMV/L420H

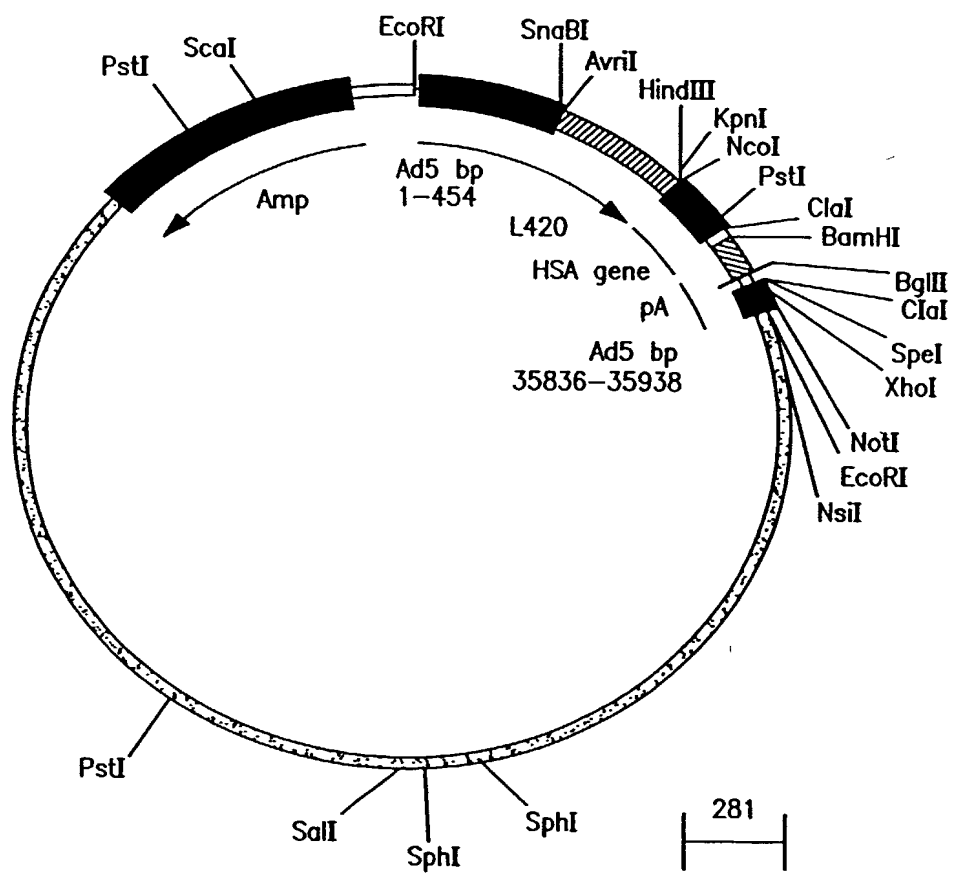


FIG. 24

Construction of pWE/AdΔ5'

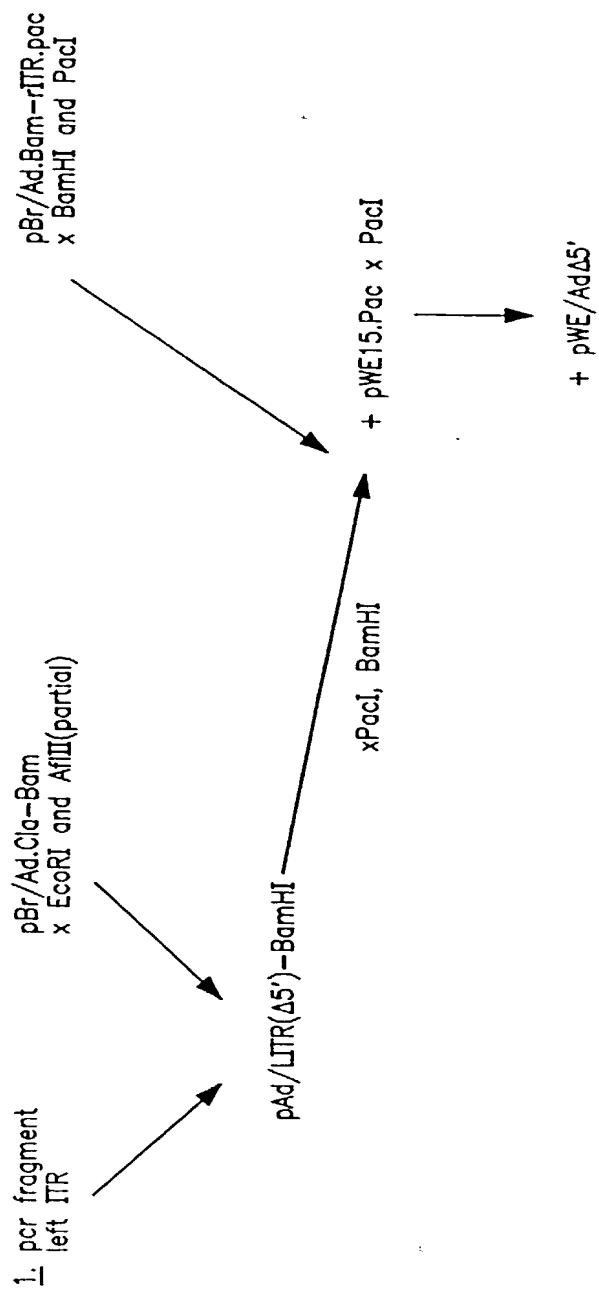
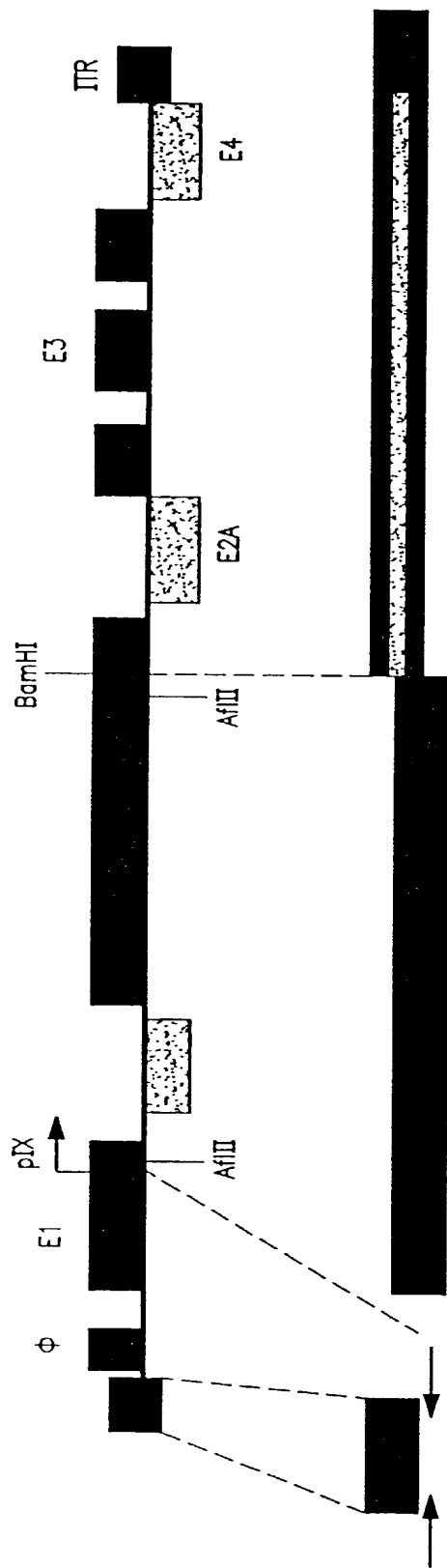


FIG. 25

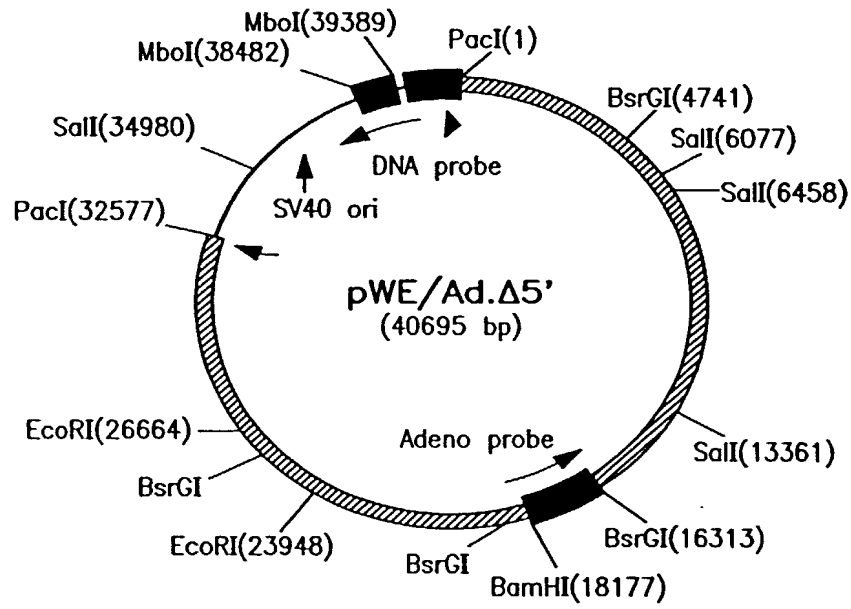


FIG. 26A

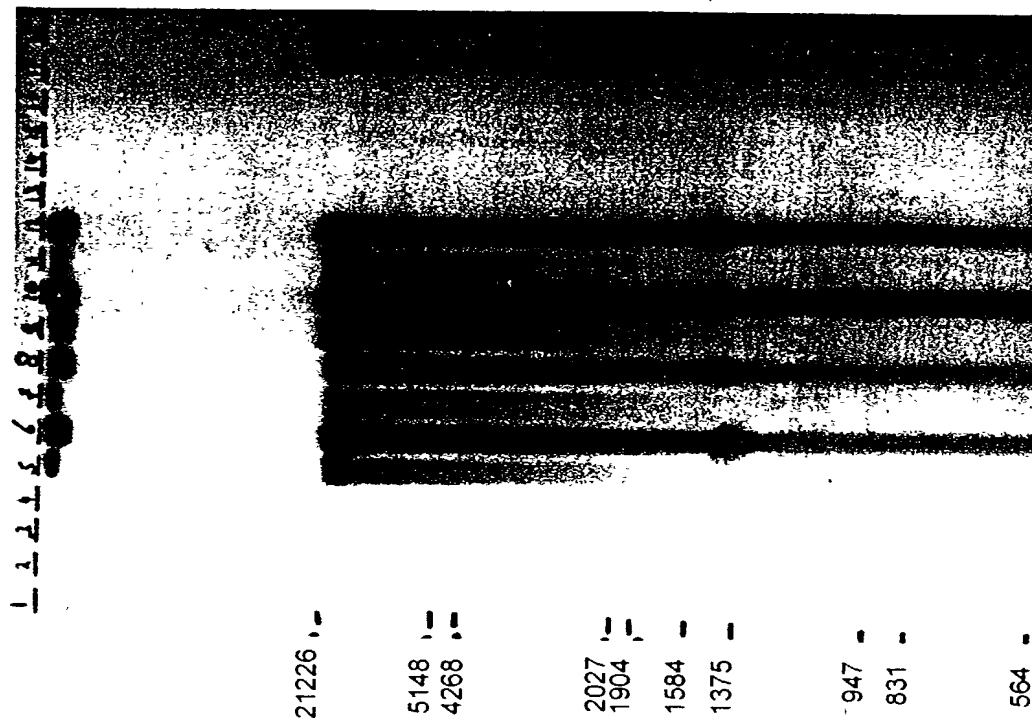


FIG. 26B

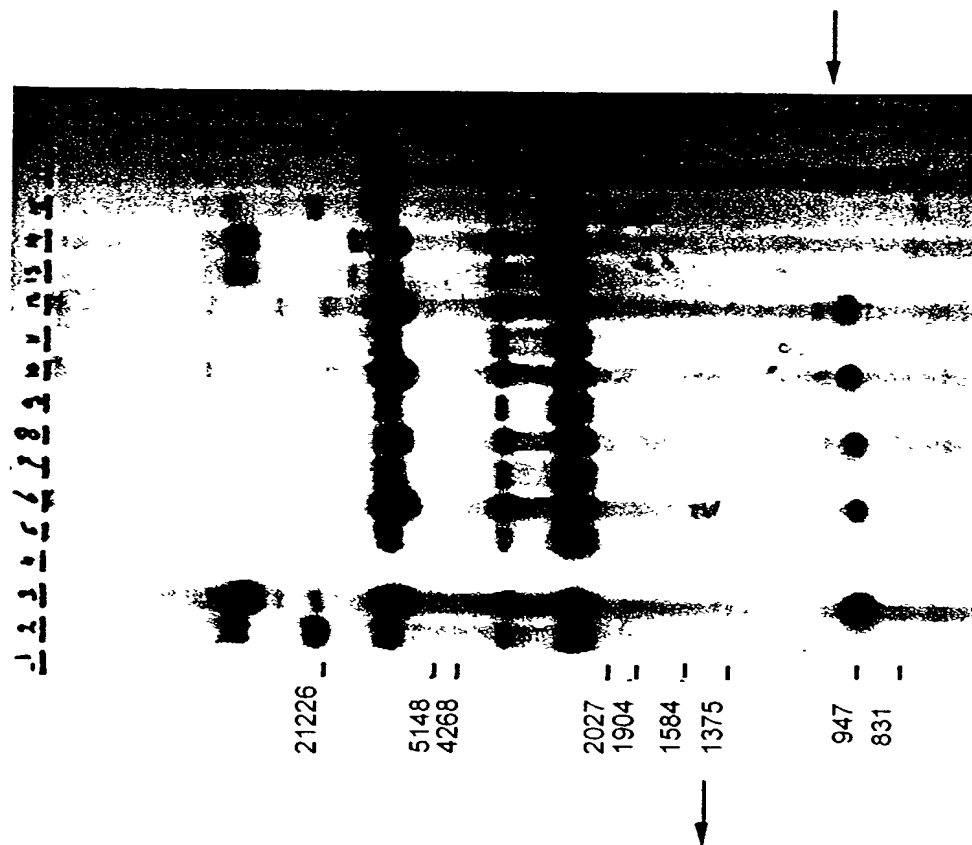
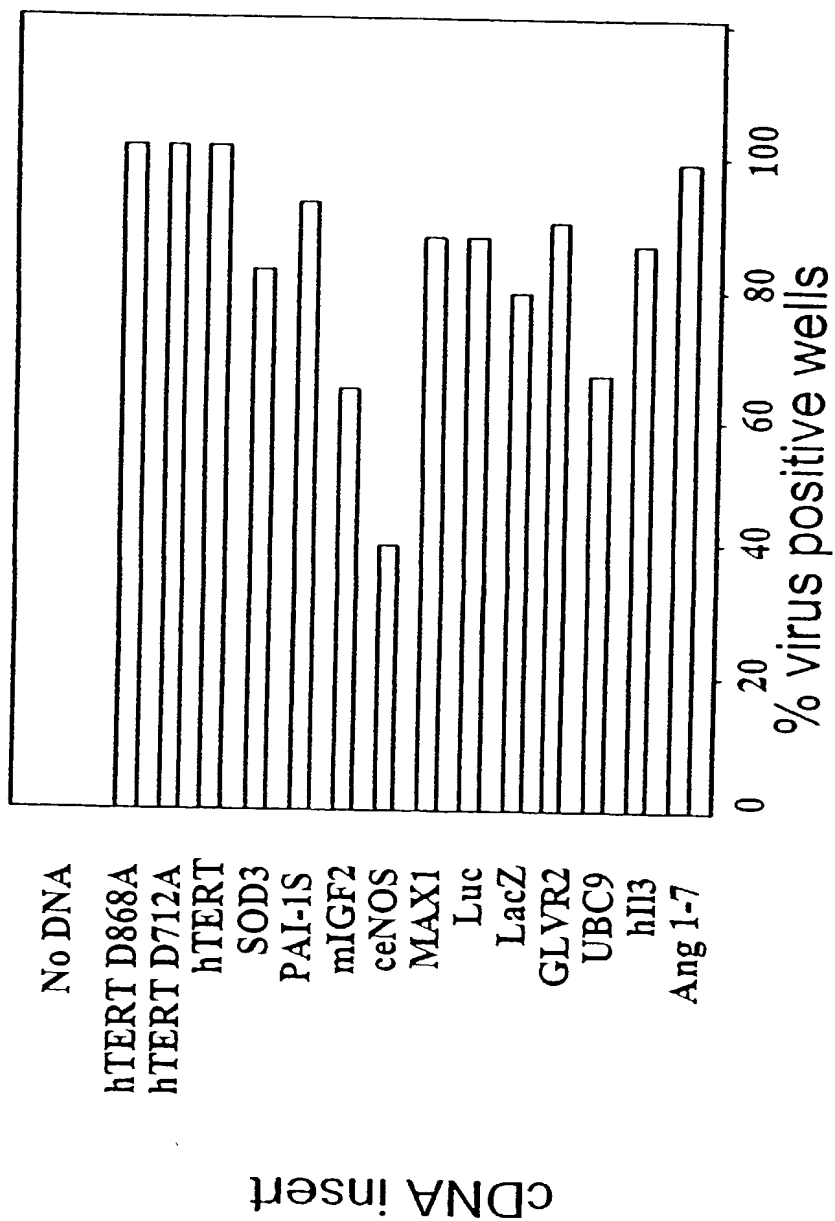


FIG. 26C

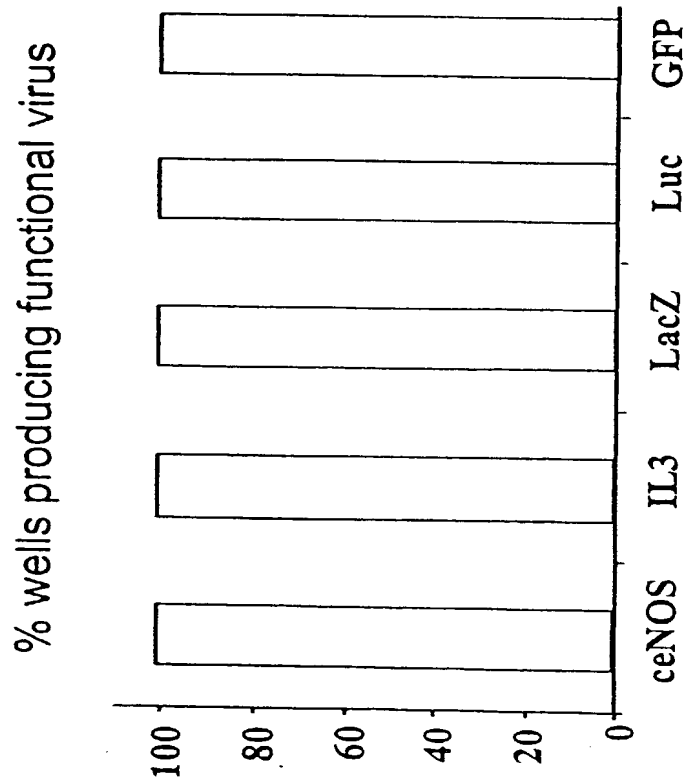


Average percentage CPE efficiency: 86 %

FIG. 27

| Gene | Insert kb | Average titer 0.8 ±0.7 x 10 ⁹ pfu/ml |
|---------------|-----------|--|
| • ceNOS | 3.6 | |
| • hTERT | 3.5 | |
| • hTERT D712A | 3.5 | |
| • lacZ | 3.2 | |
| • hCAT1 | 2.2 | |
| • GLVR2 | 2.0 | |
| • Luc | 1.7 | |
| • SOD3 | 1.4 | |
| • MAX1 | .550 | |
| • hVEGF121 | .511 | |
| • hIL3 | .434 | |
| • UBC9 | .412 | |
| • ANG1-7 | .104 | |

FIG. 28



| Gene | Number of CPE+ wells |
|-------|----------------------|
| ceNOS | 19/19 |
| IL3 | 7/7 |
| lacZ | 36/36 |
| Luc | 40/40 |
| GFP | 48/48 |

| Gene | Number of plaques |
|-------|-------------------|
| ceNOS | 9/9 |
| IL3 | 9/9 |
| lacZ | 40/40 |
| Luc | 9/9 |
| EGFP | IP |
| GLVR2 | 9/9 |

FIG. 29

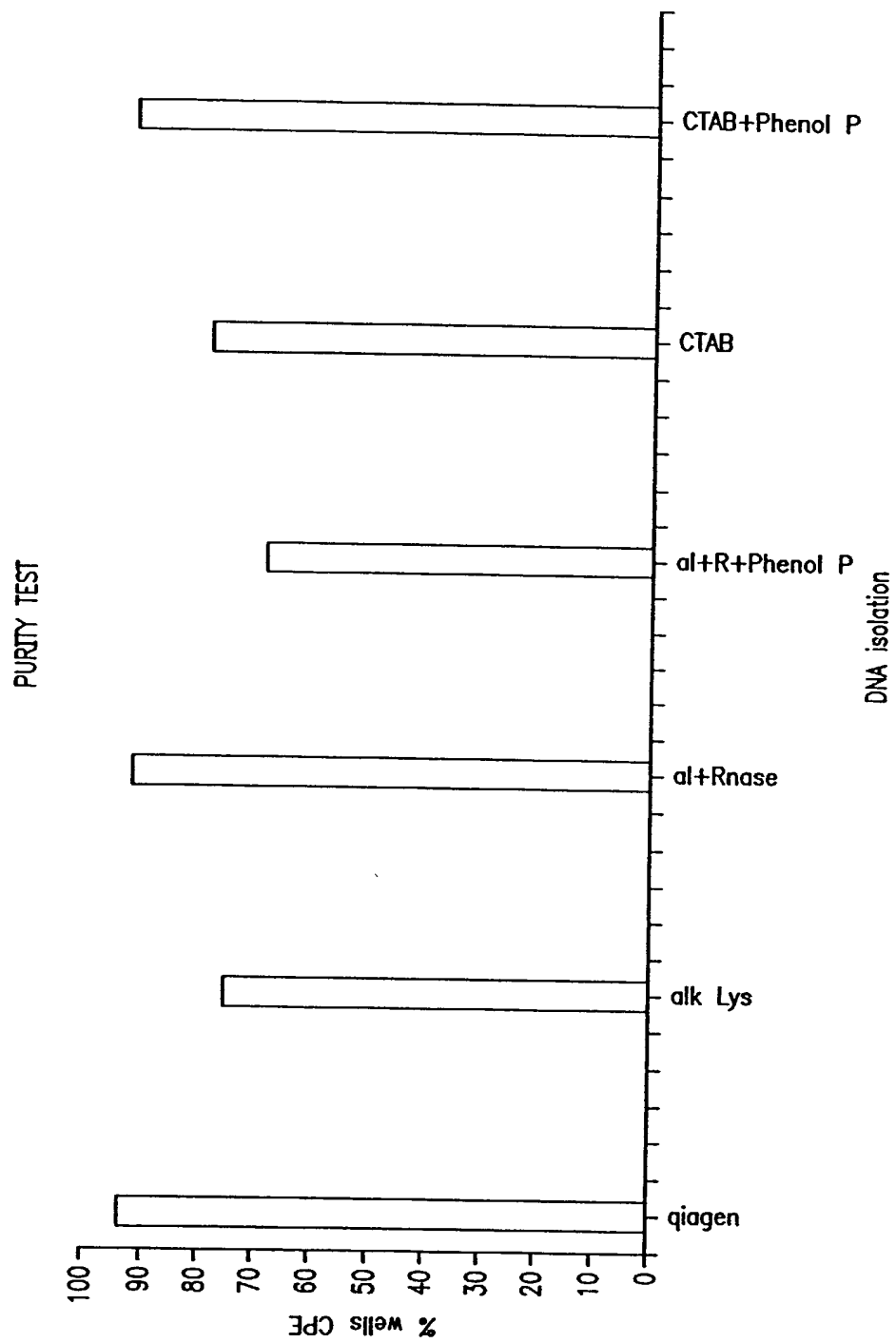


FIG. 30

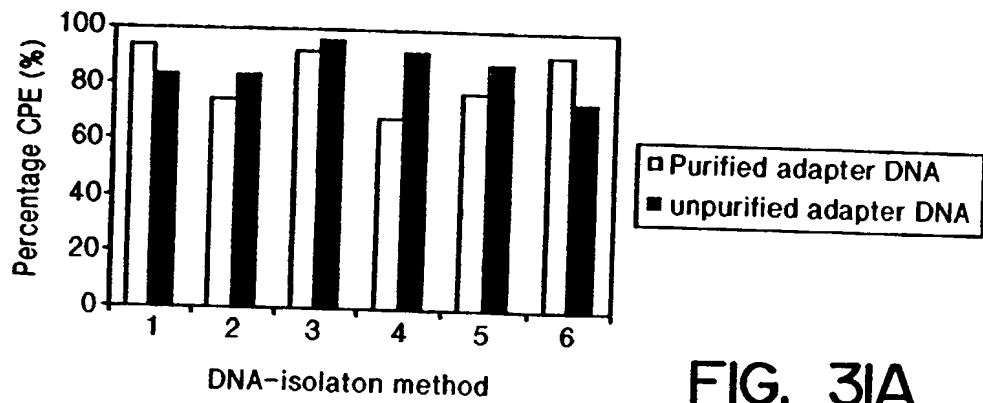


FIG. 3IA

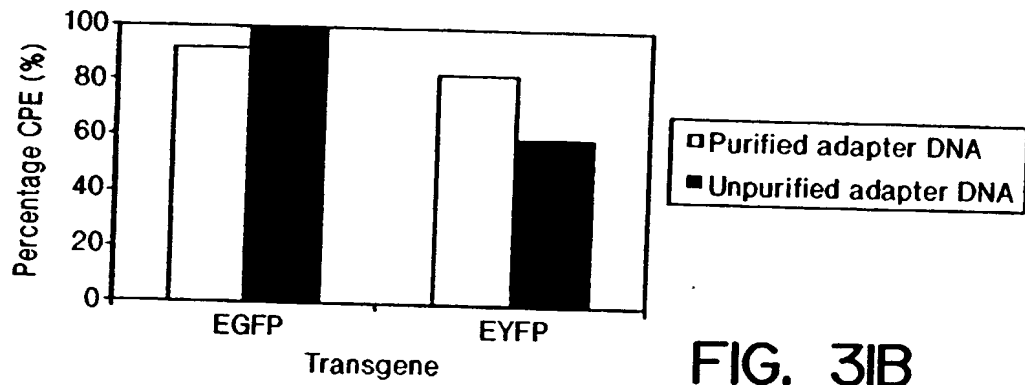


FIG. 3IB

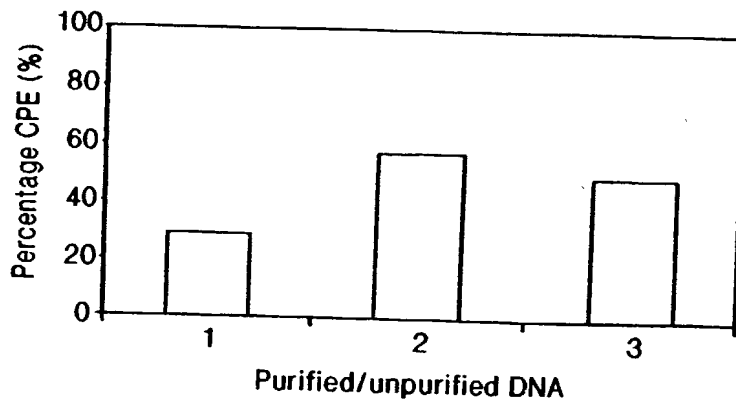
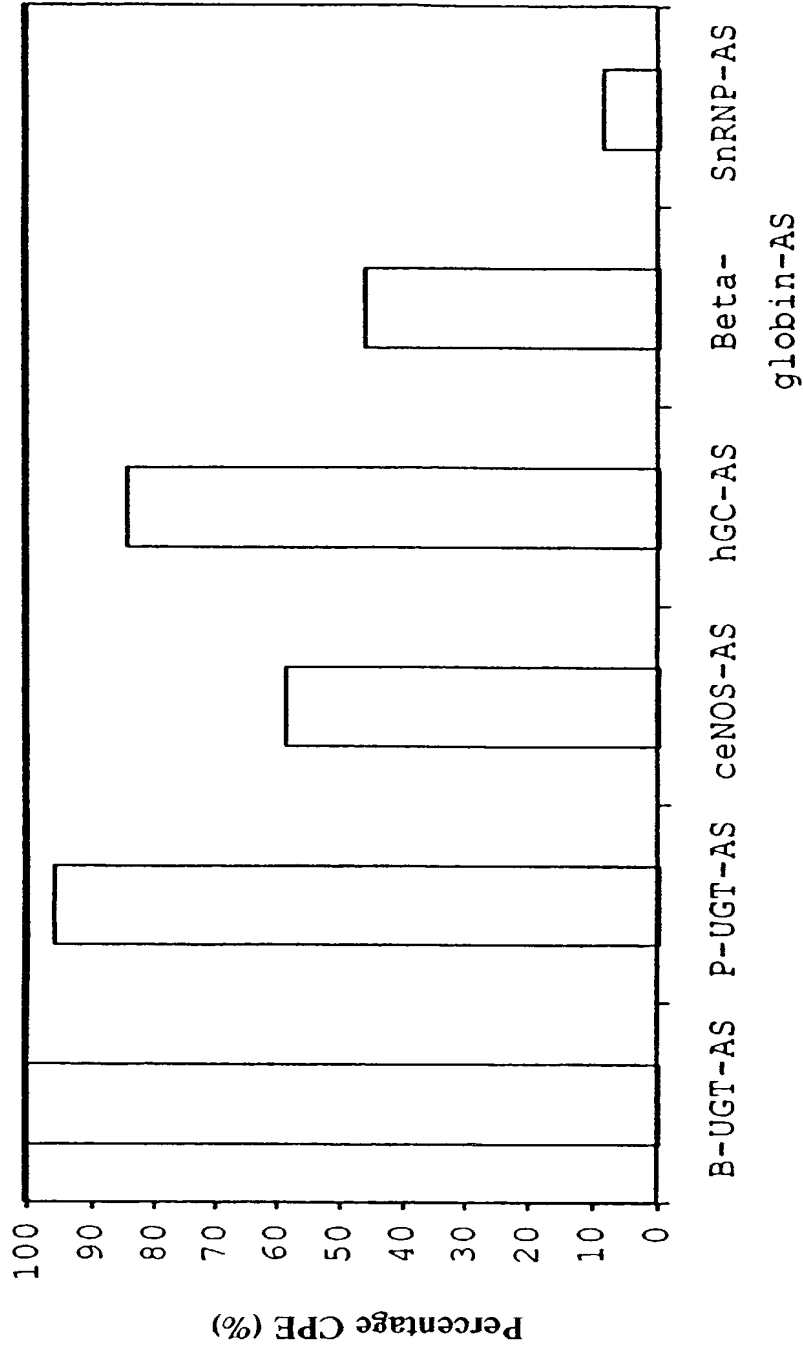


FIG. 3IC



AS-virus

FIG. 33

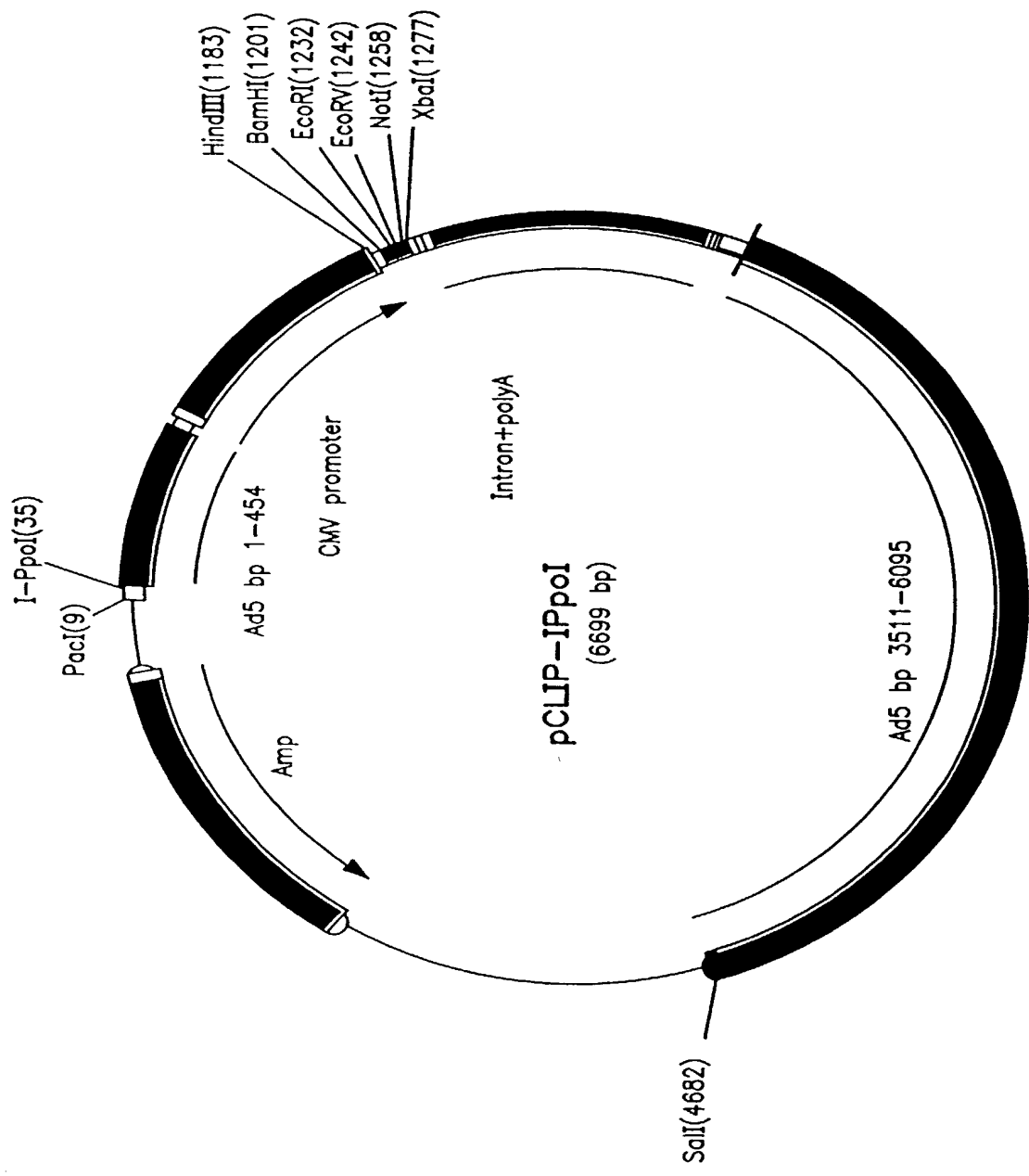


FIG. 34A

FIG. 34A

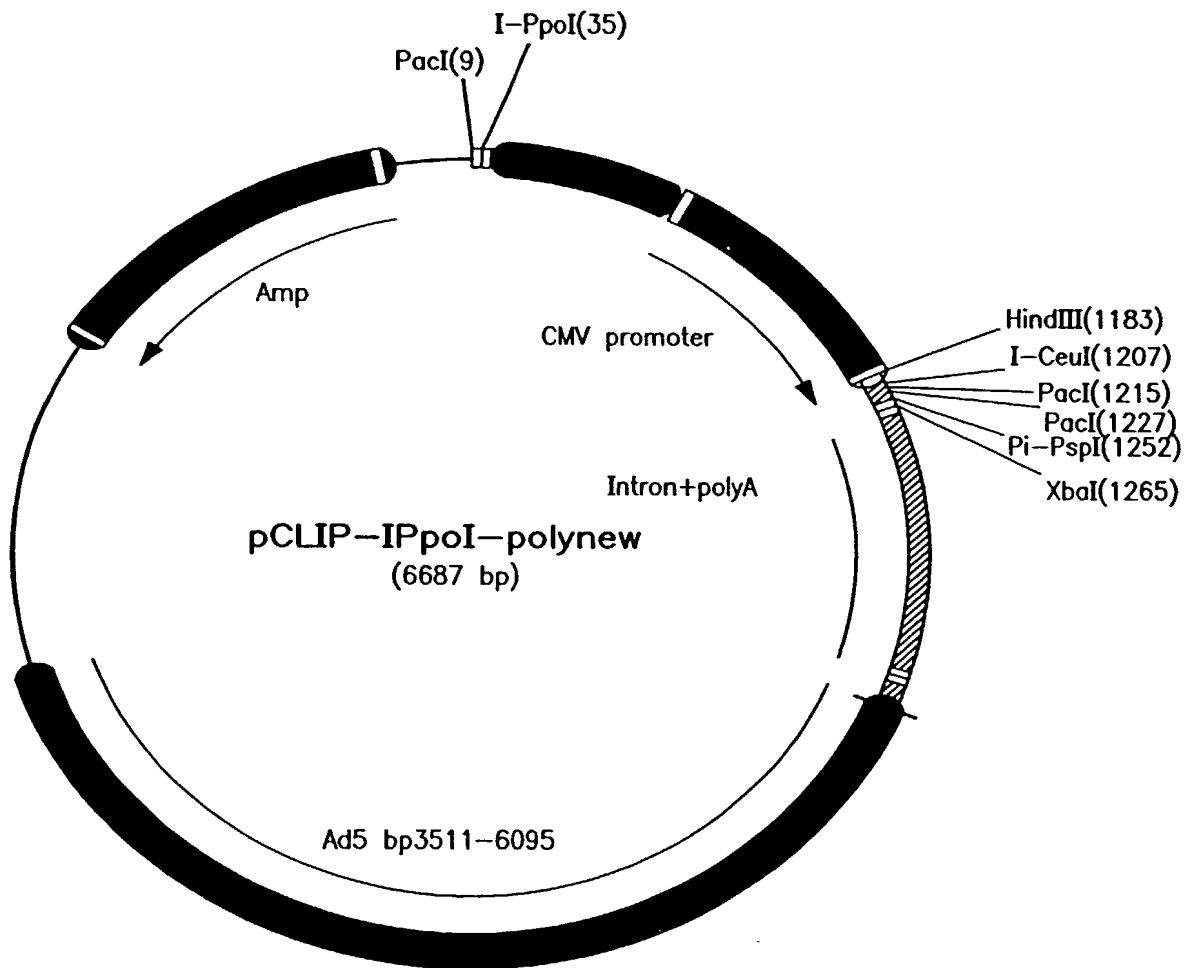


FIG. 34B

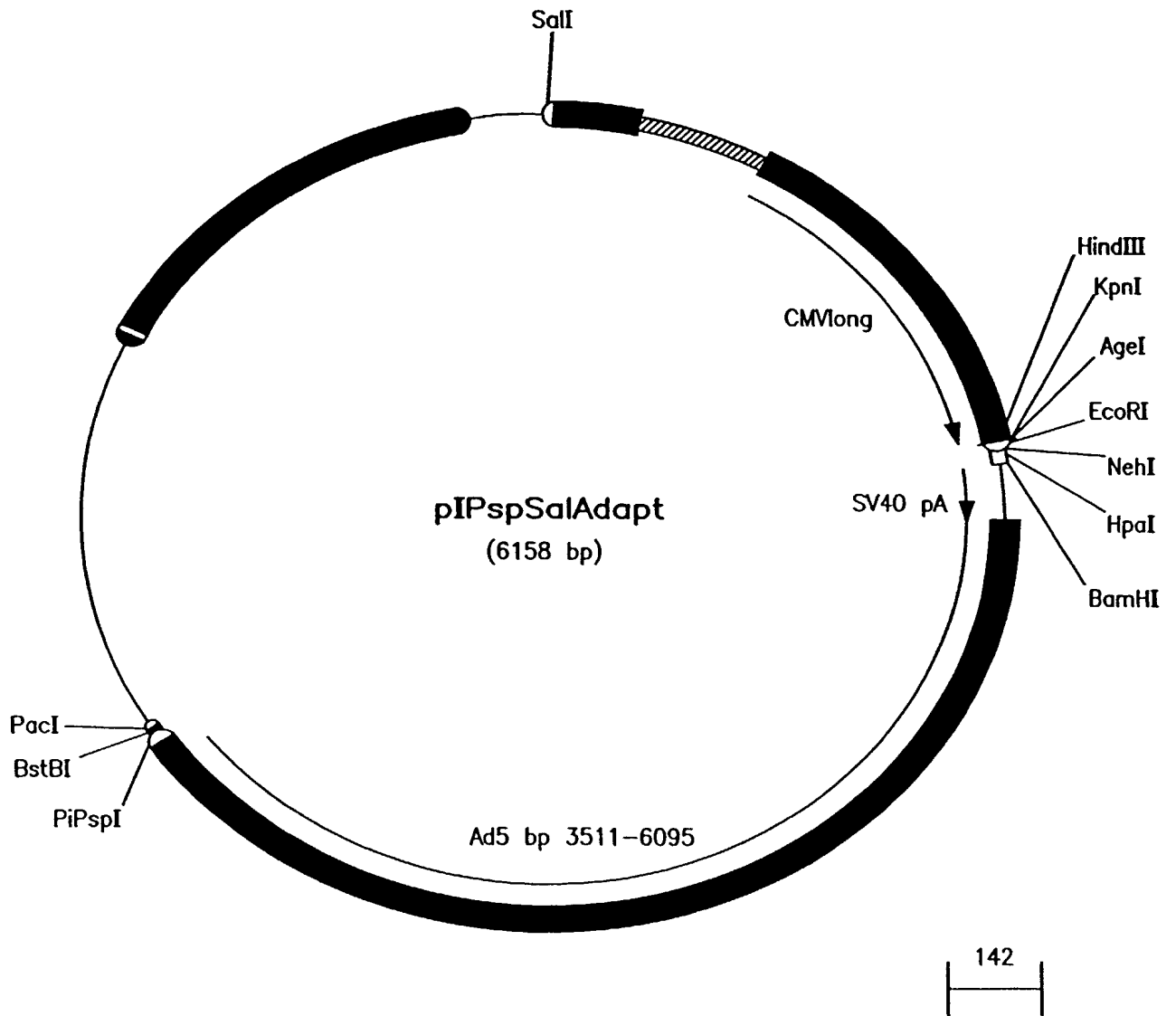


FIG. 34C

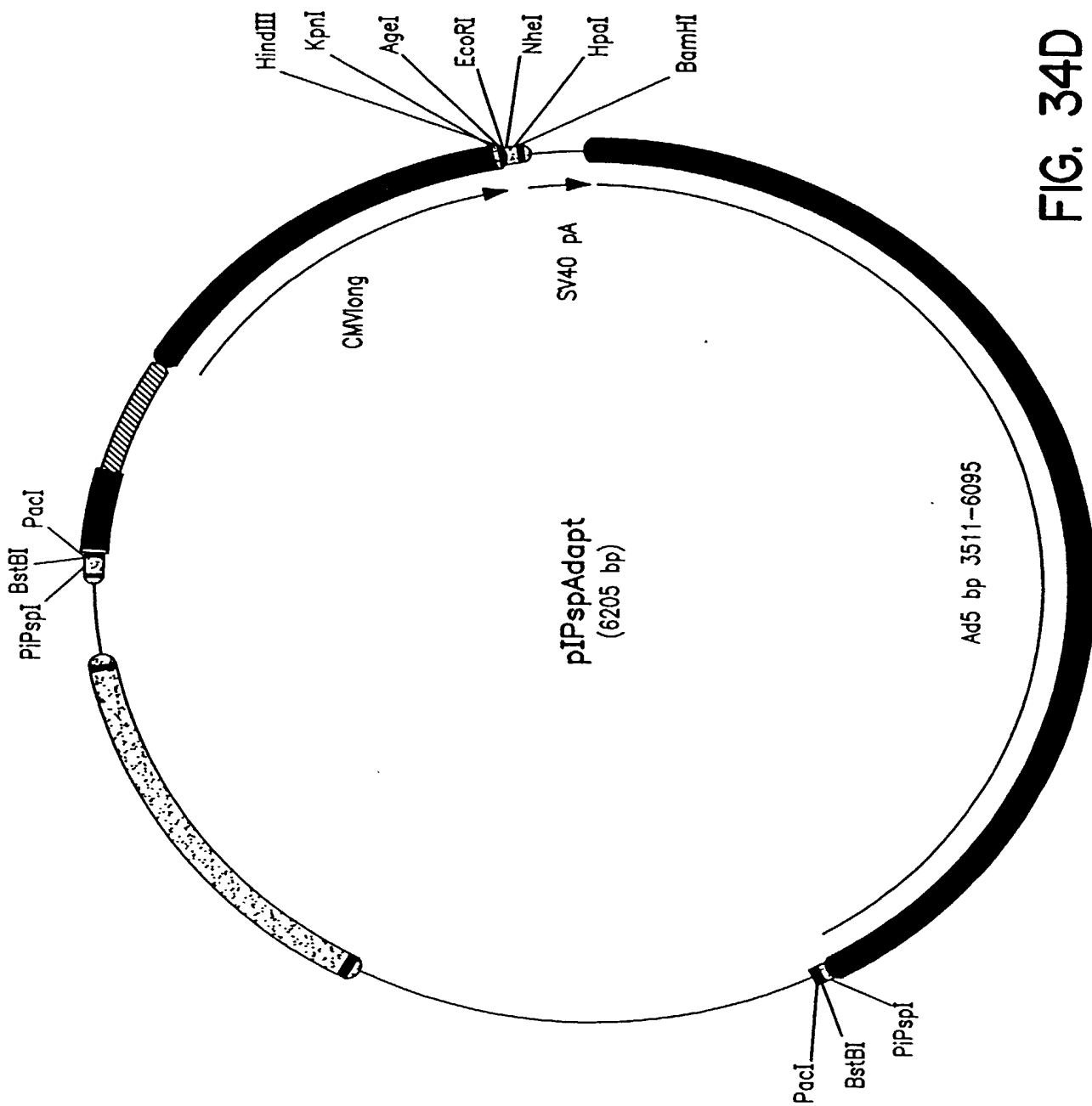


FIG. 34D

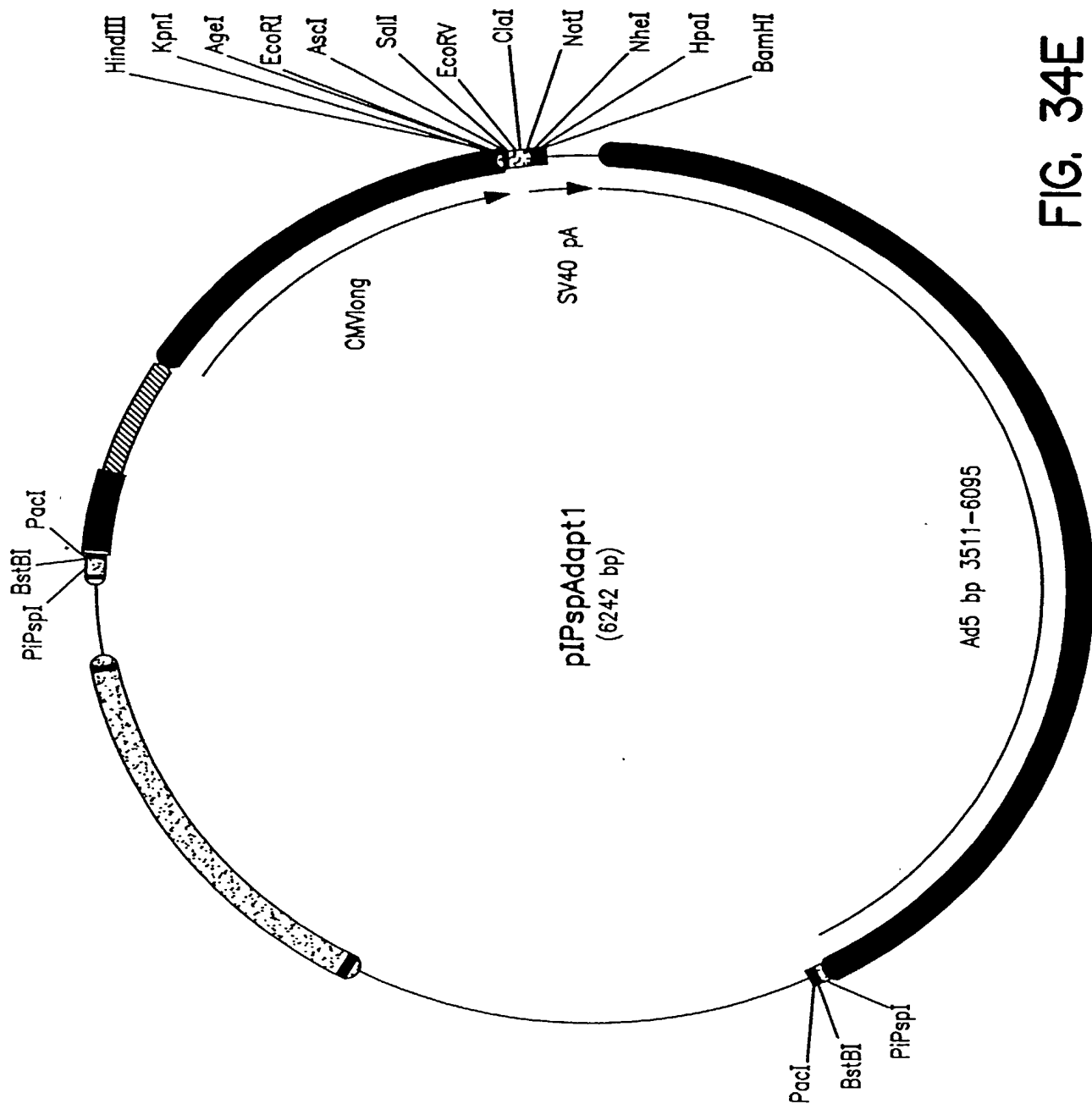


FIG. 34E

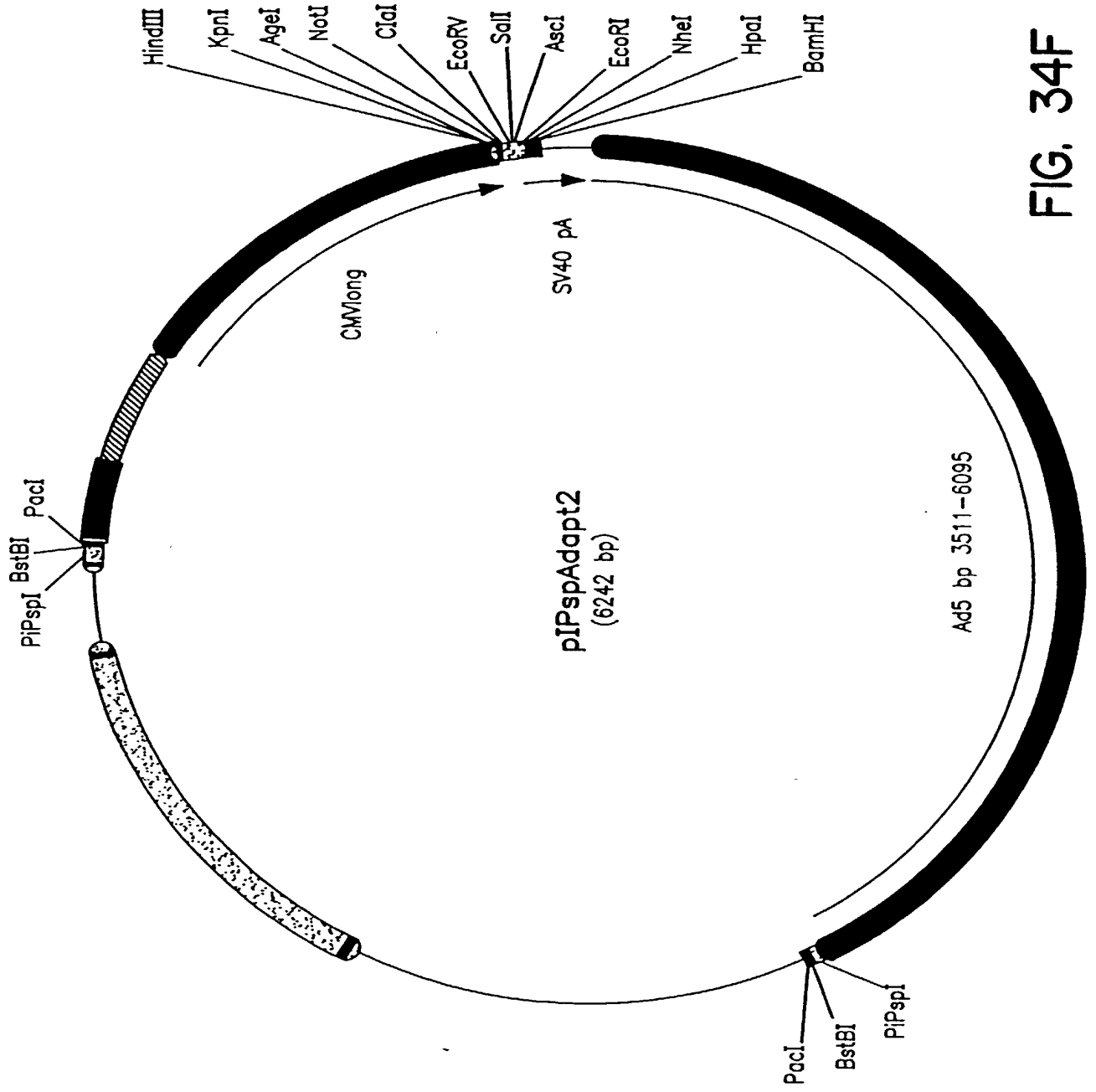


FIG. 34F

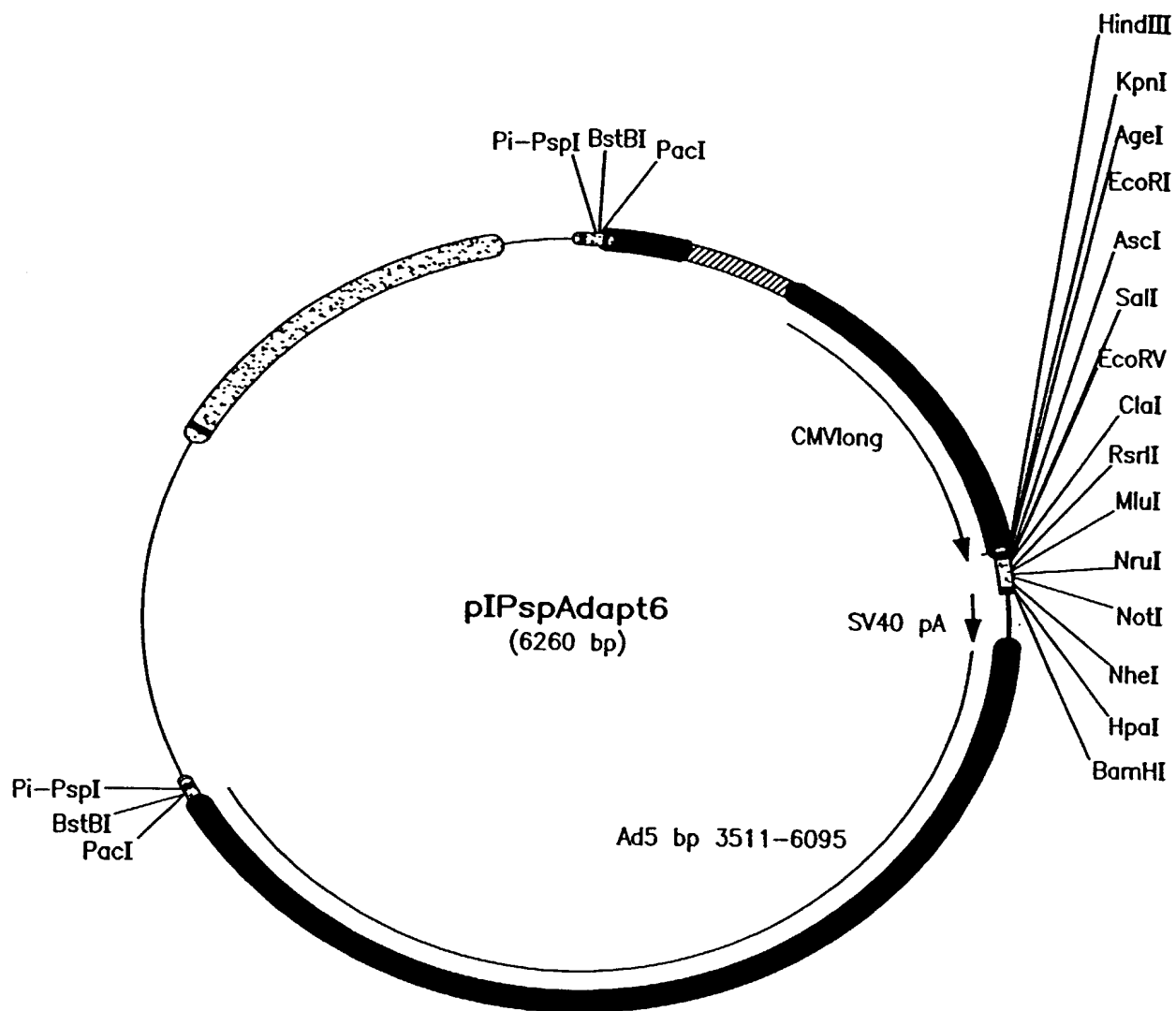
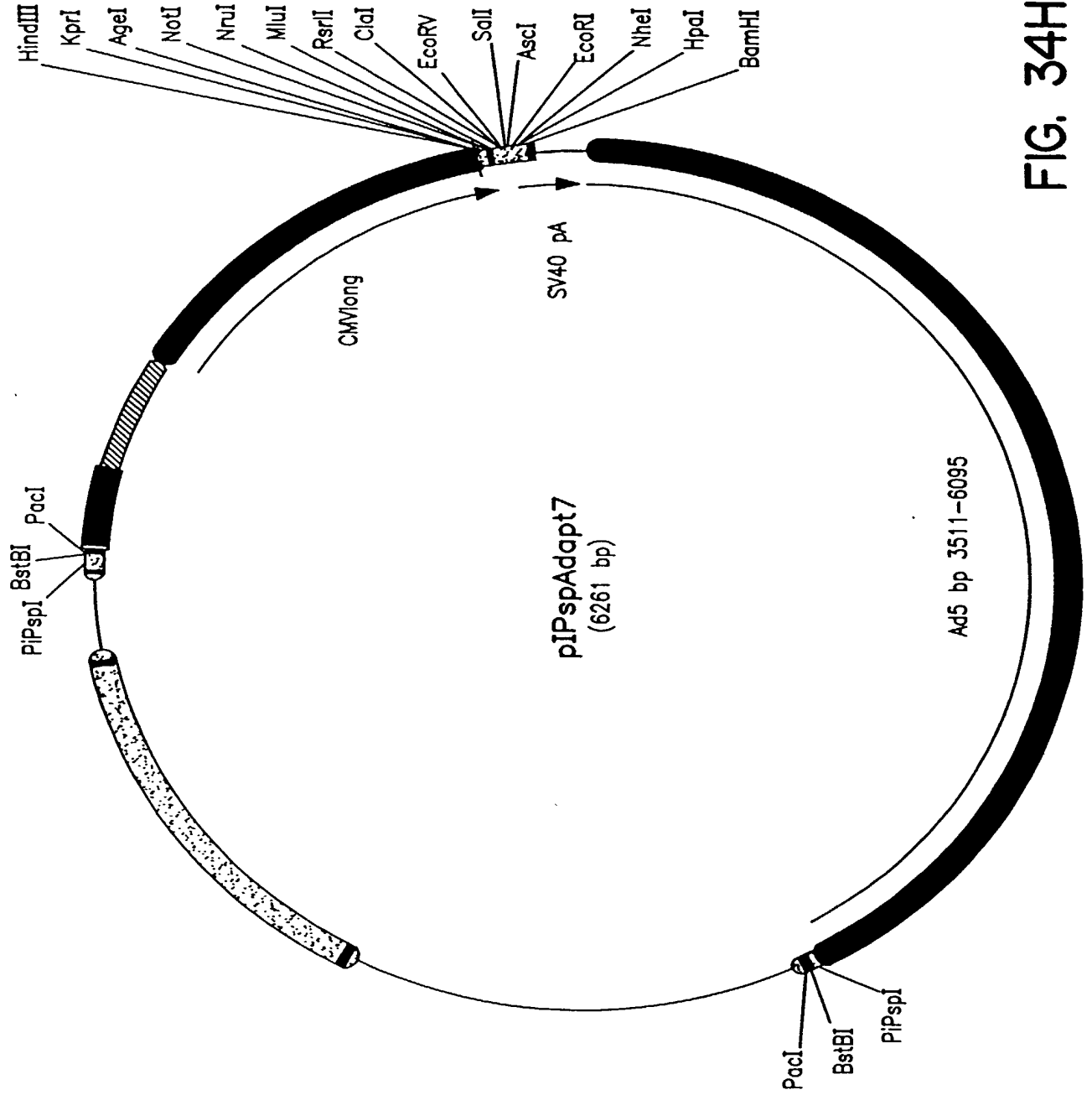


FIG. 34G



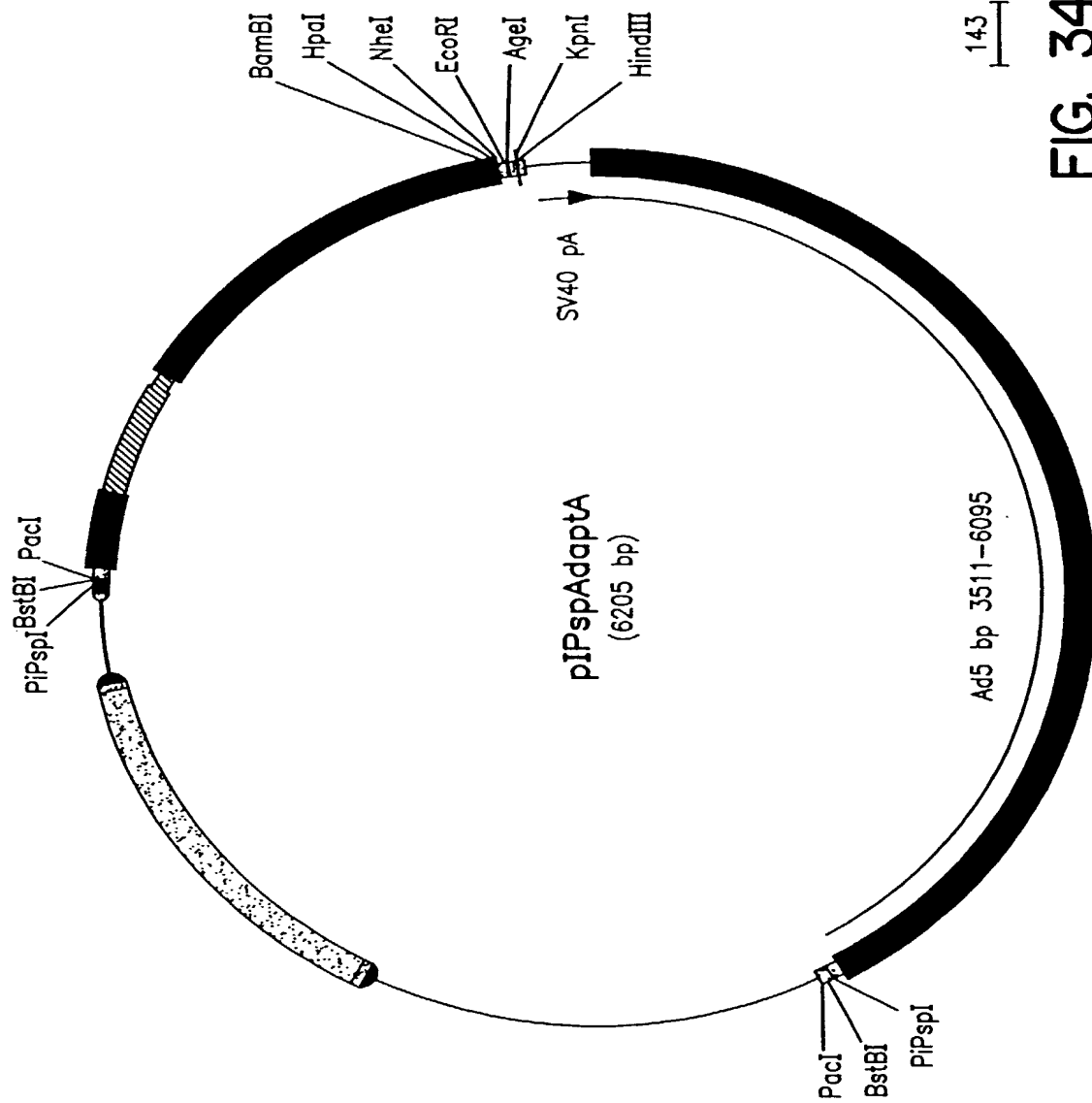


FIG. 34 I

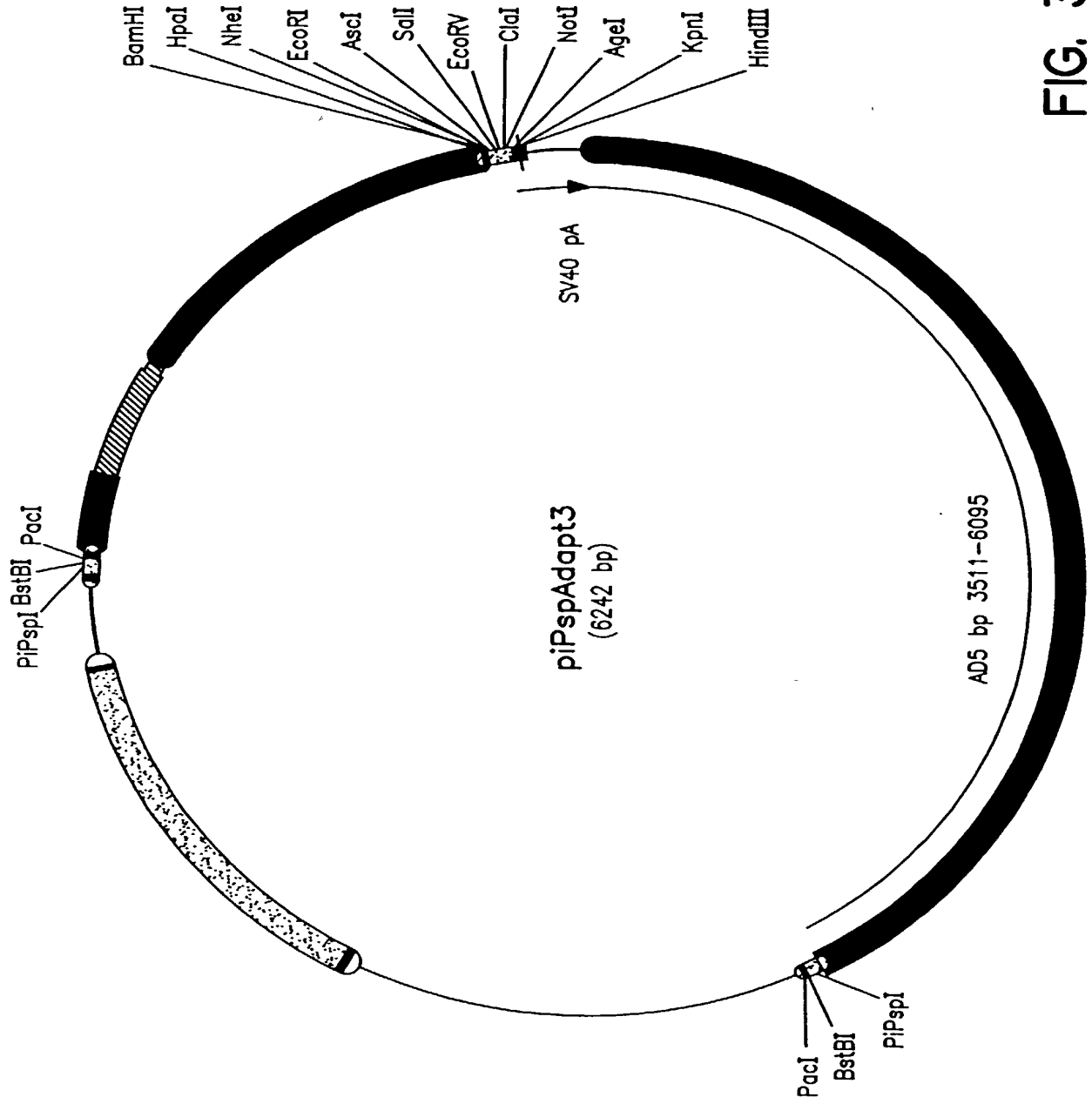


FIG. 34J

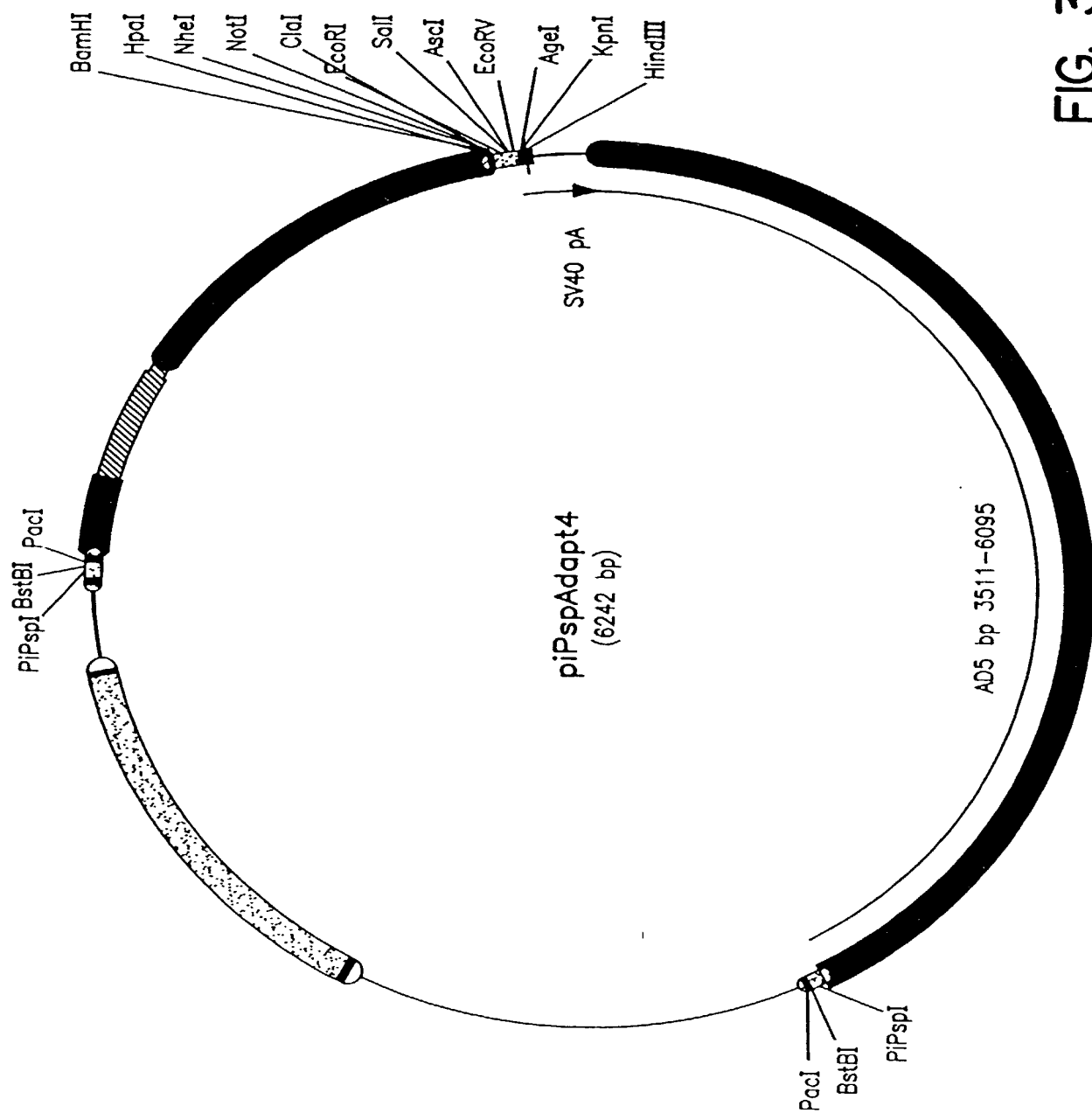


FIG. 34K

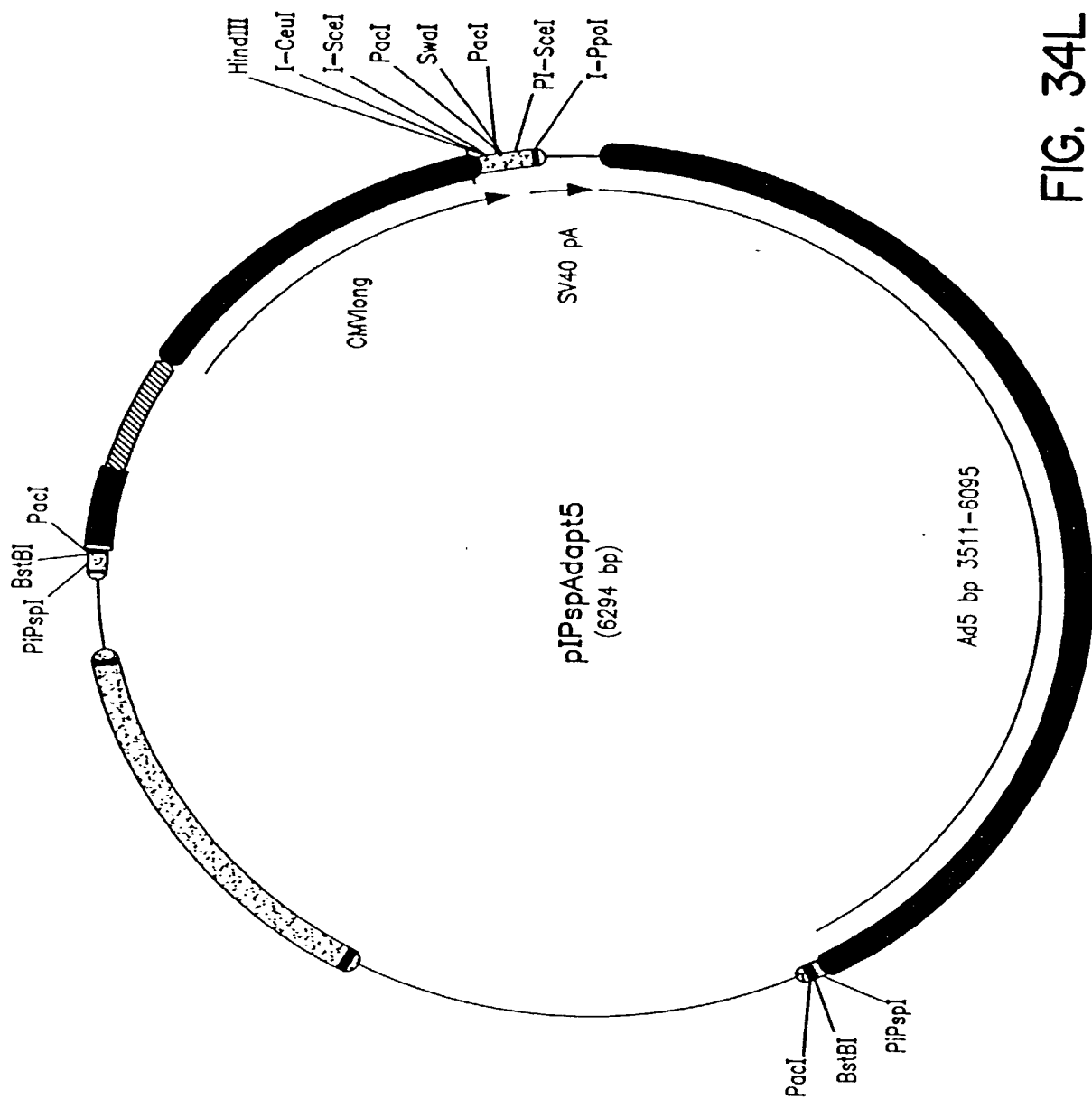


FIG. 34L

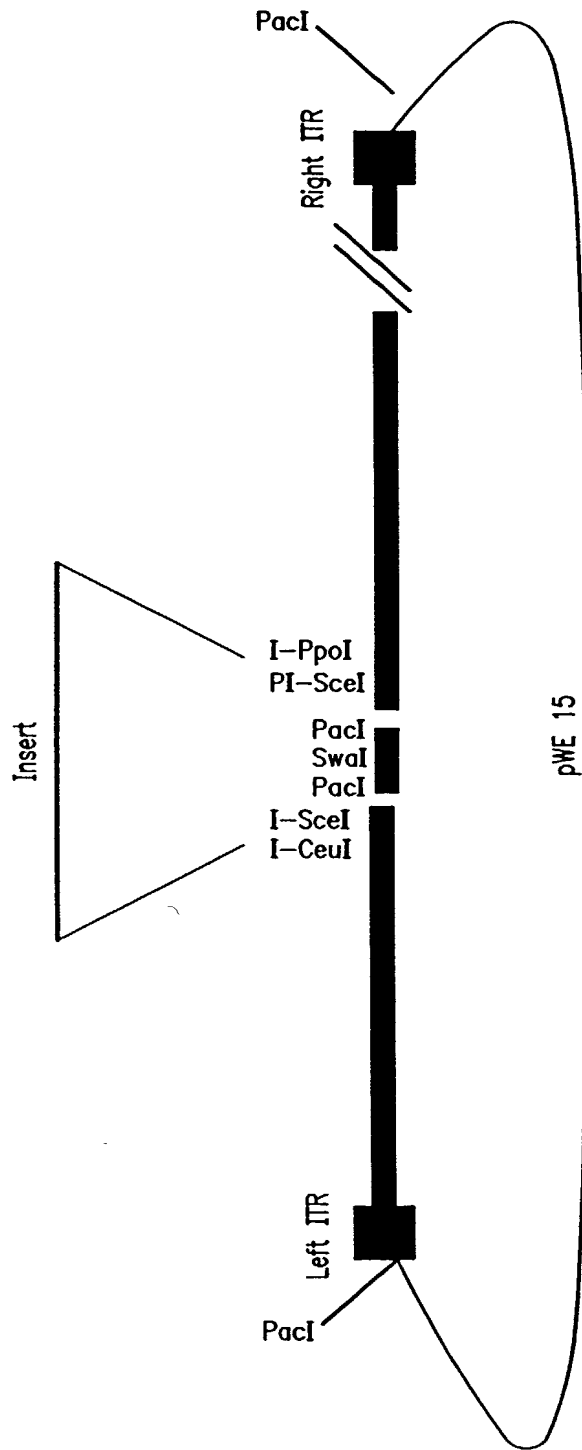


FIG. 34M

Relative amounts of wells with CPE after transfection of
PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

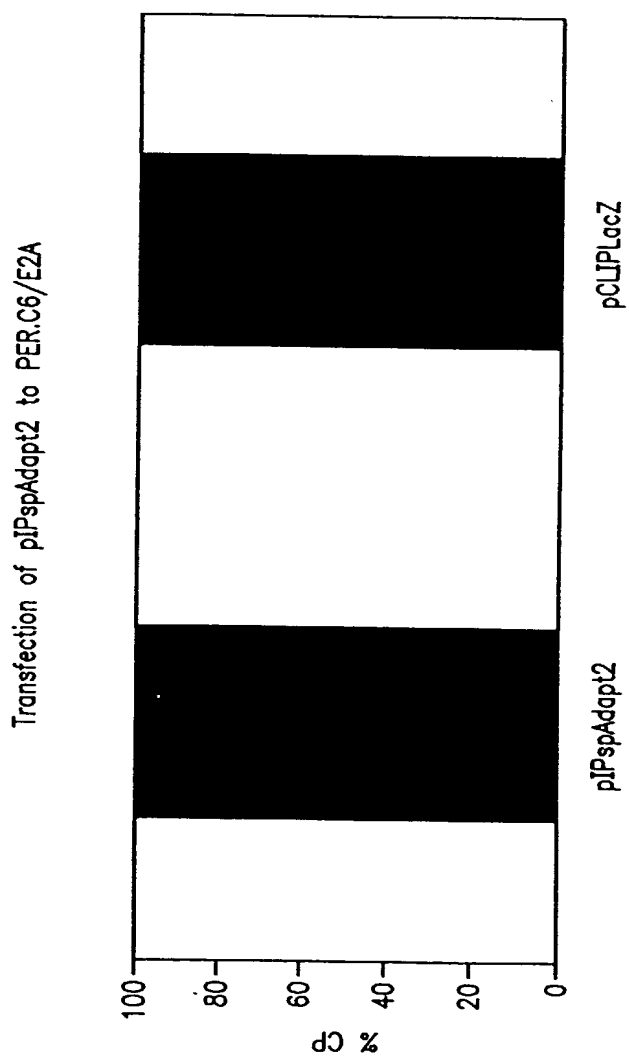


FIG. 34N

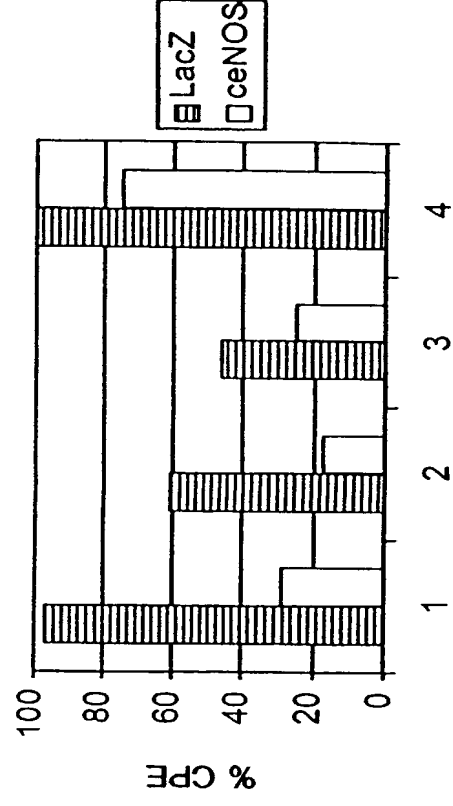


FIG. 35

FIG. 35

Construction total Adeno cDNA Library (1)

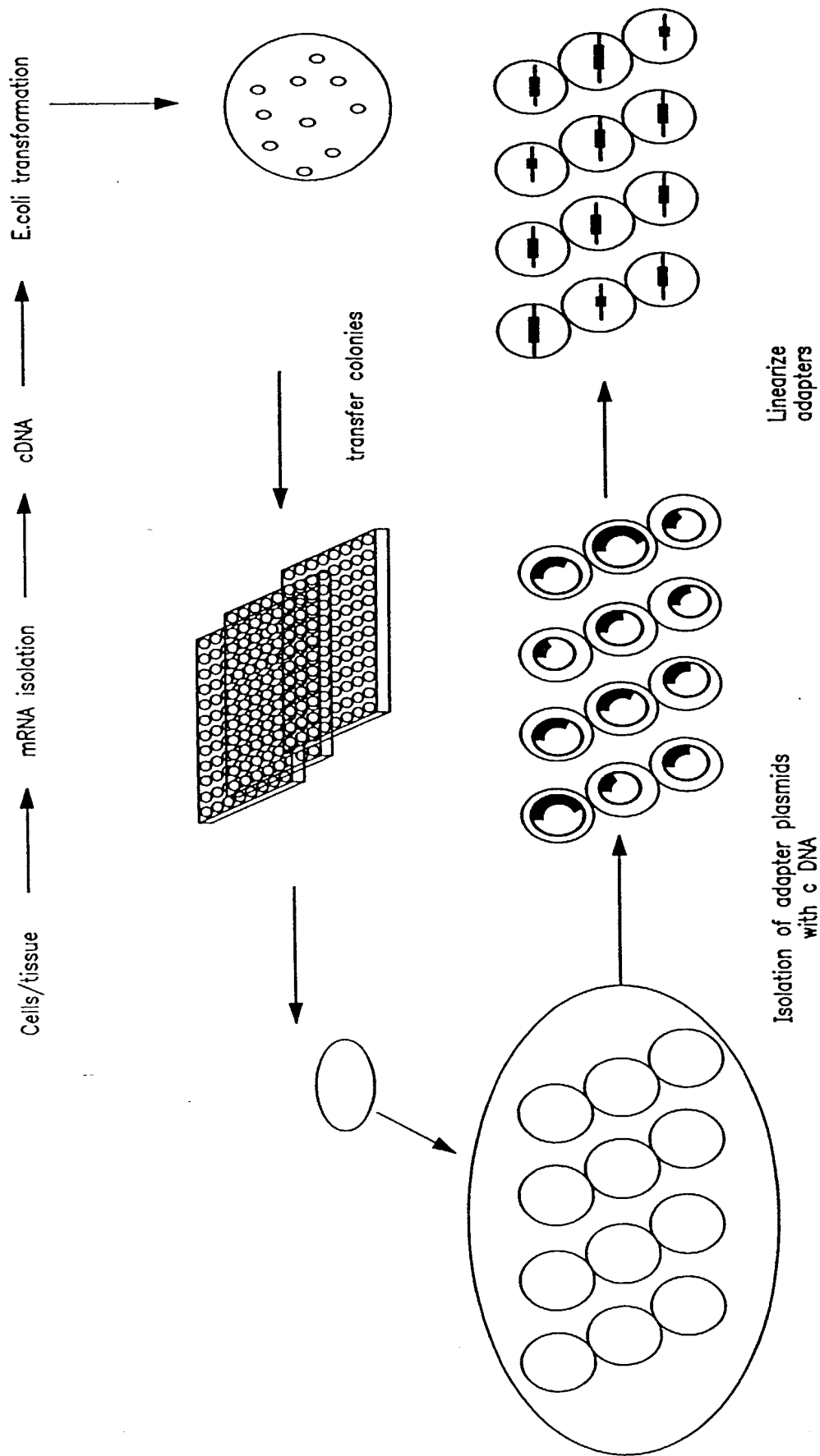


FIG. 36A

Construction total Adeno cDNA Library (II)

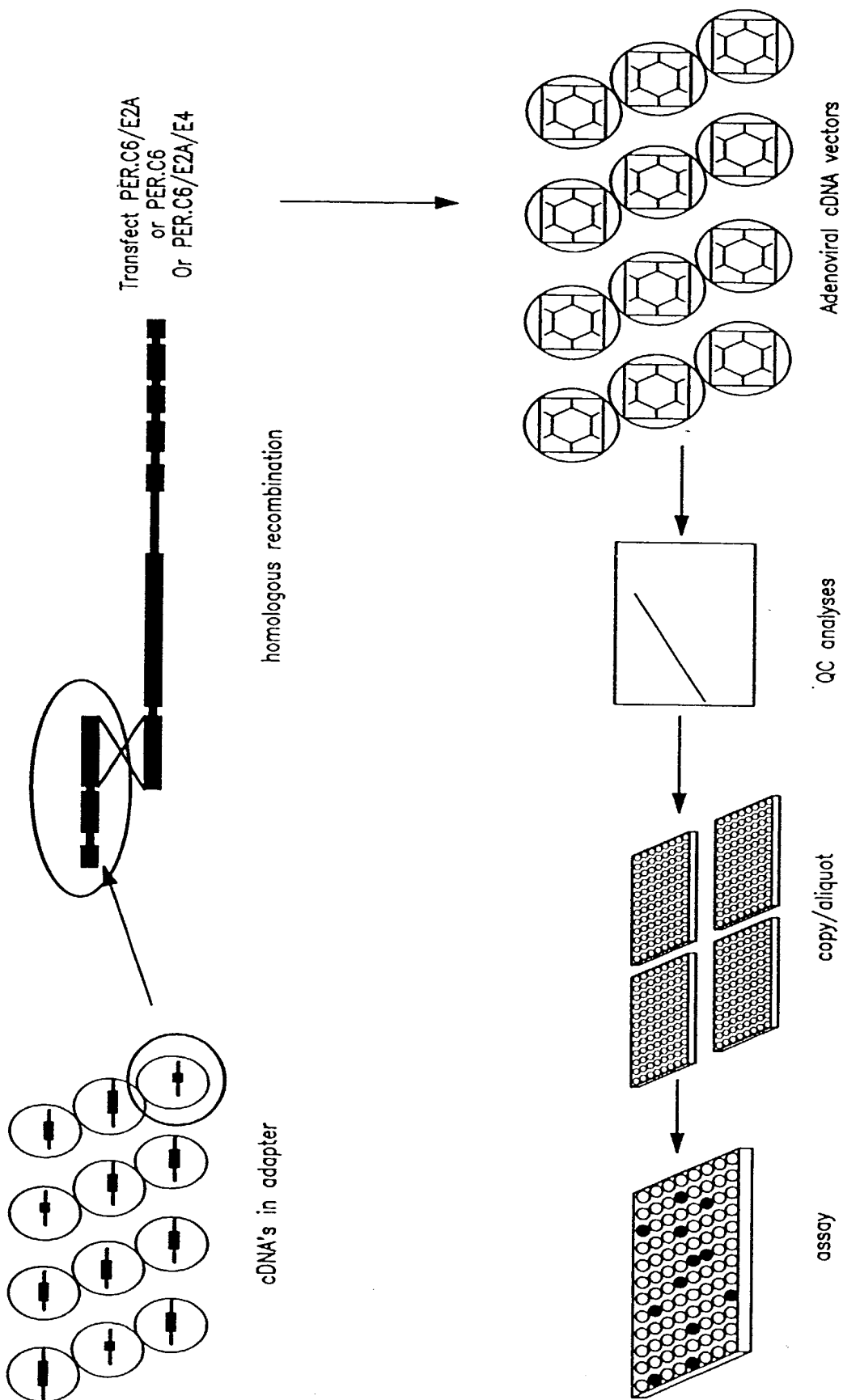


FIG. 36B

EXAMPLE 21 384 WELL PLATE IN PROGRESS

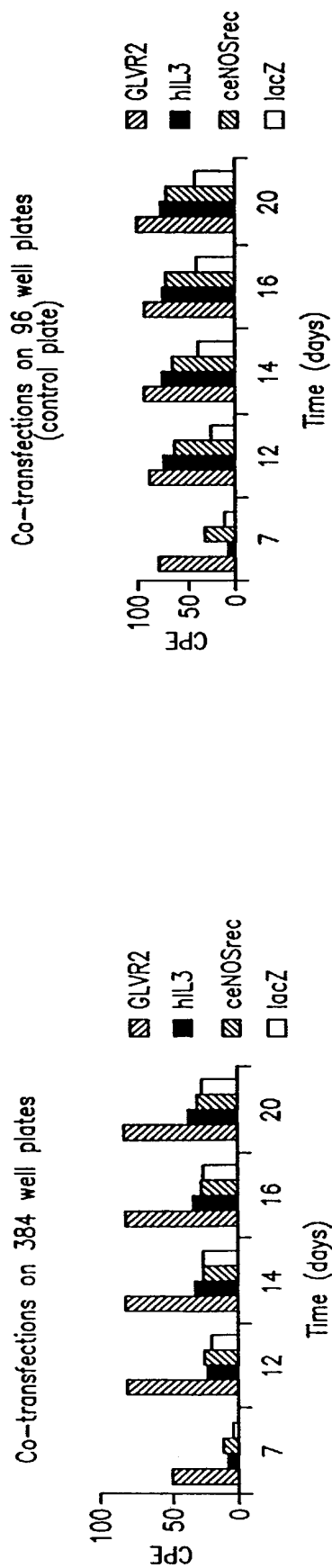


FIG. 37A

FIG. 37B

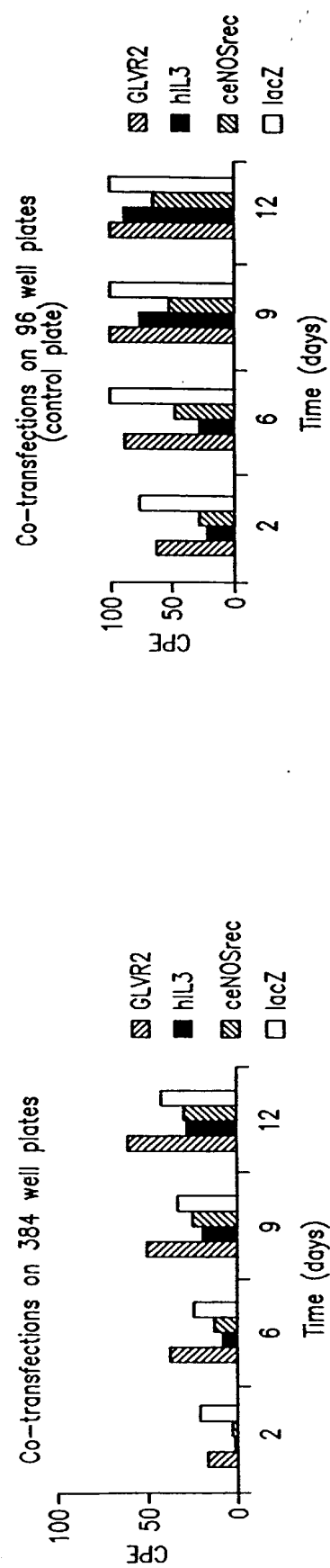


FIG. 37C

FIG. 37D

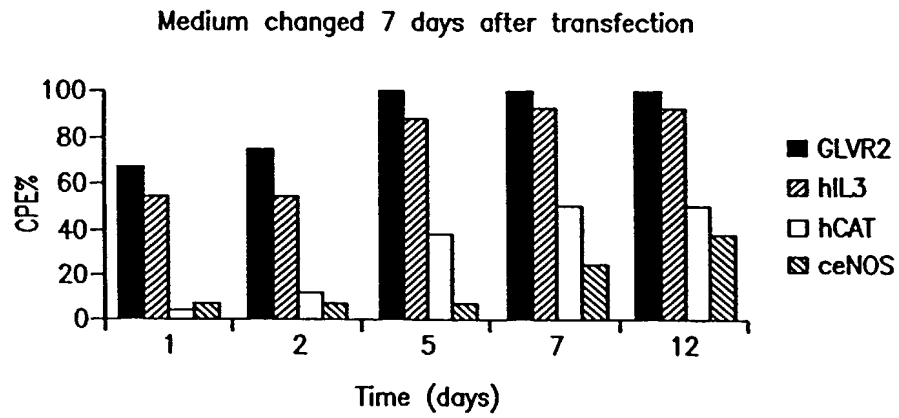


FIG. 38A

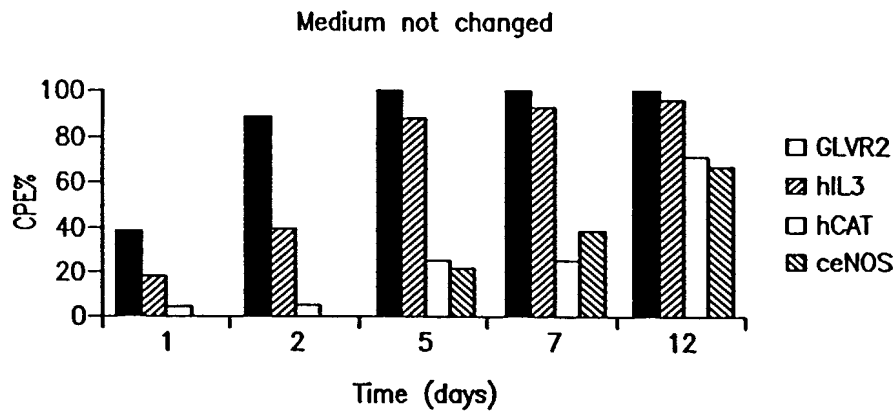


FIG. 38B

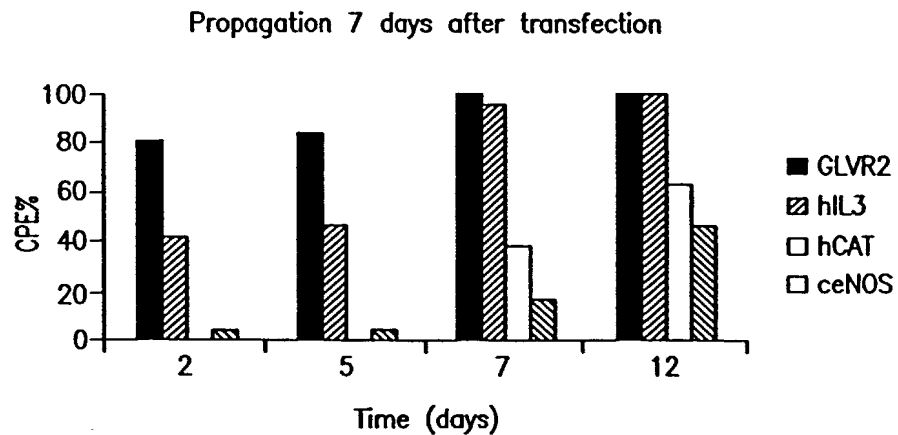


FIG. 38C

Cell titration experiment #1

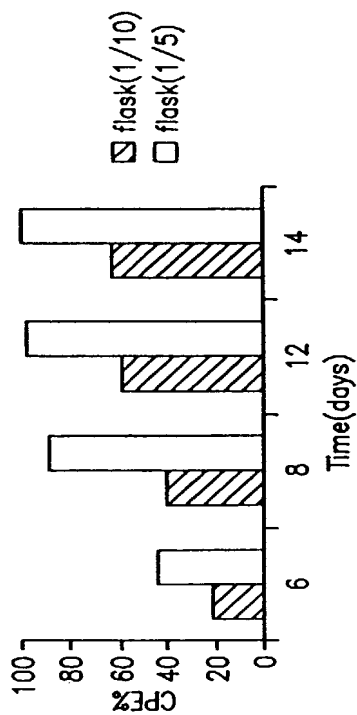


FIG. 39A

Cell titration experiment #2

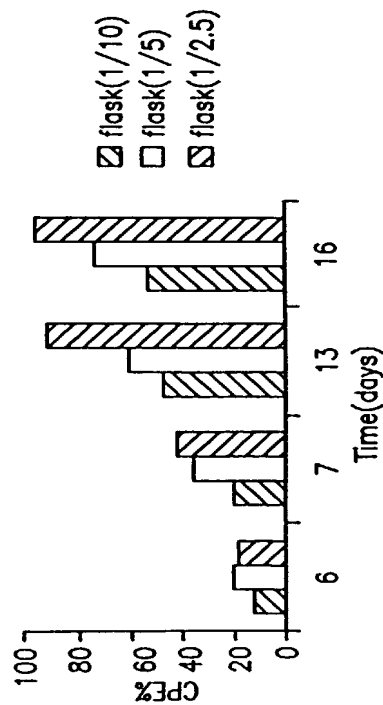


FIG. 39B

Cell titration experiment #3

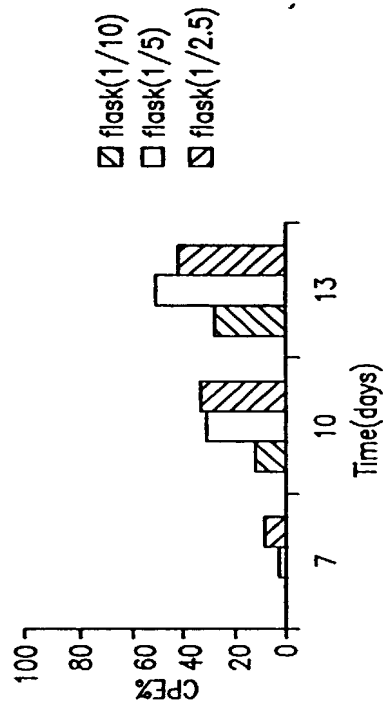


FIG. 39C

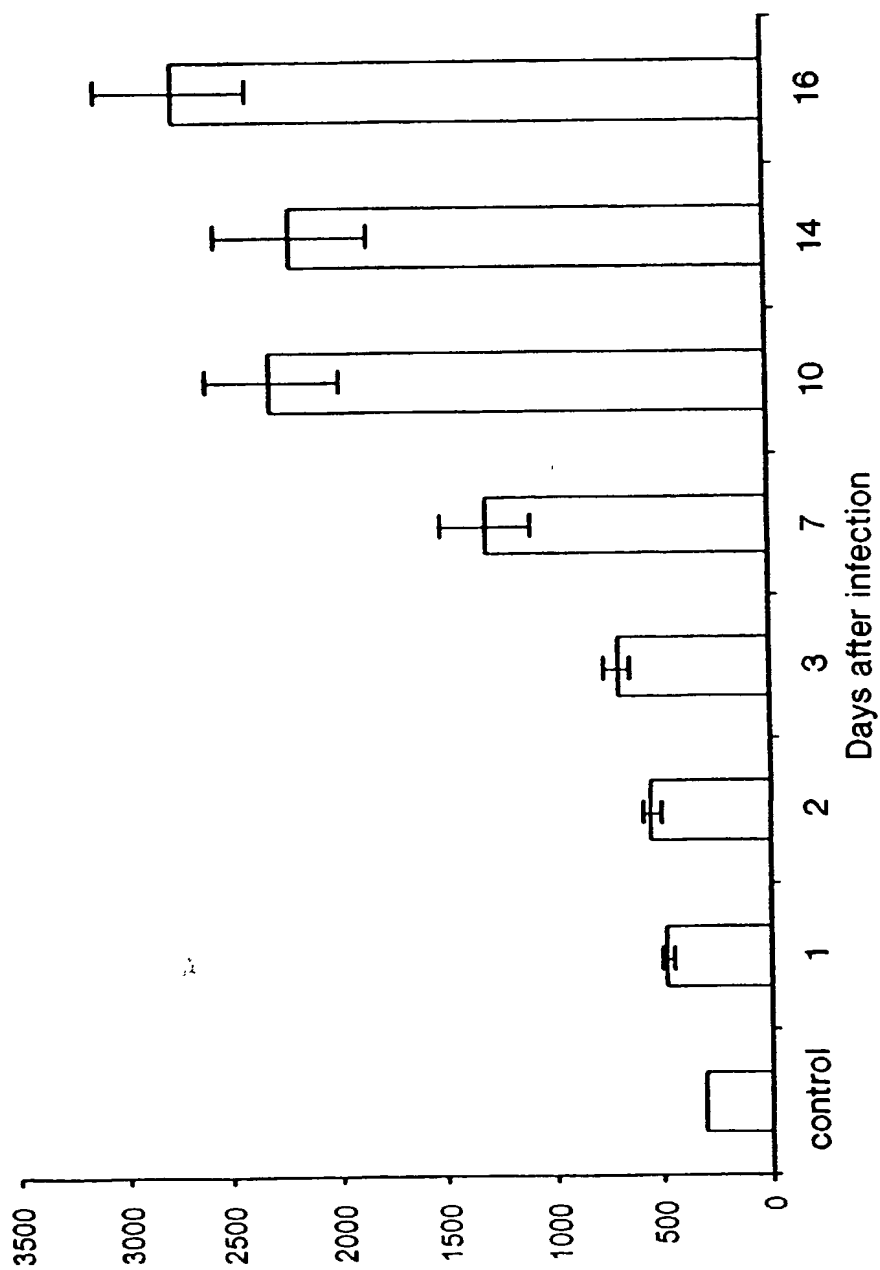
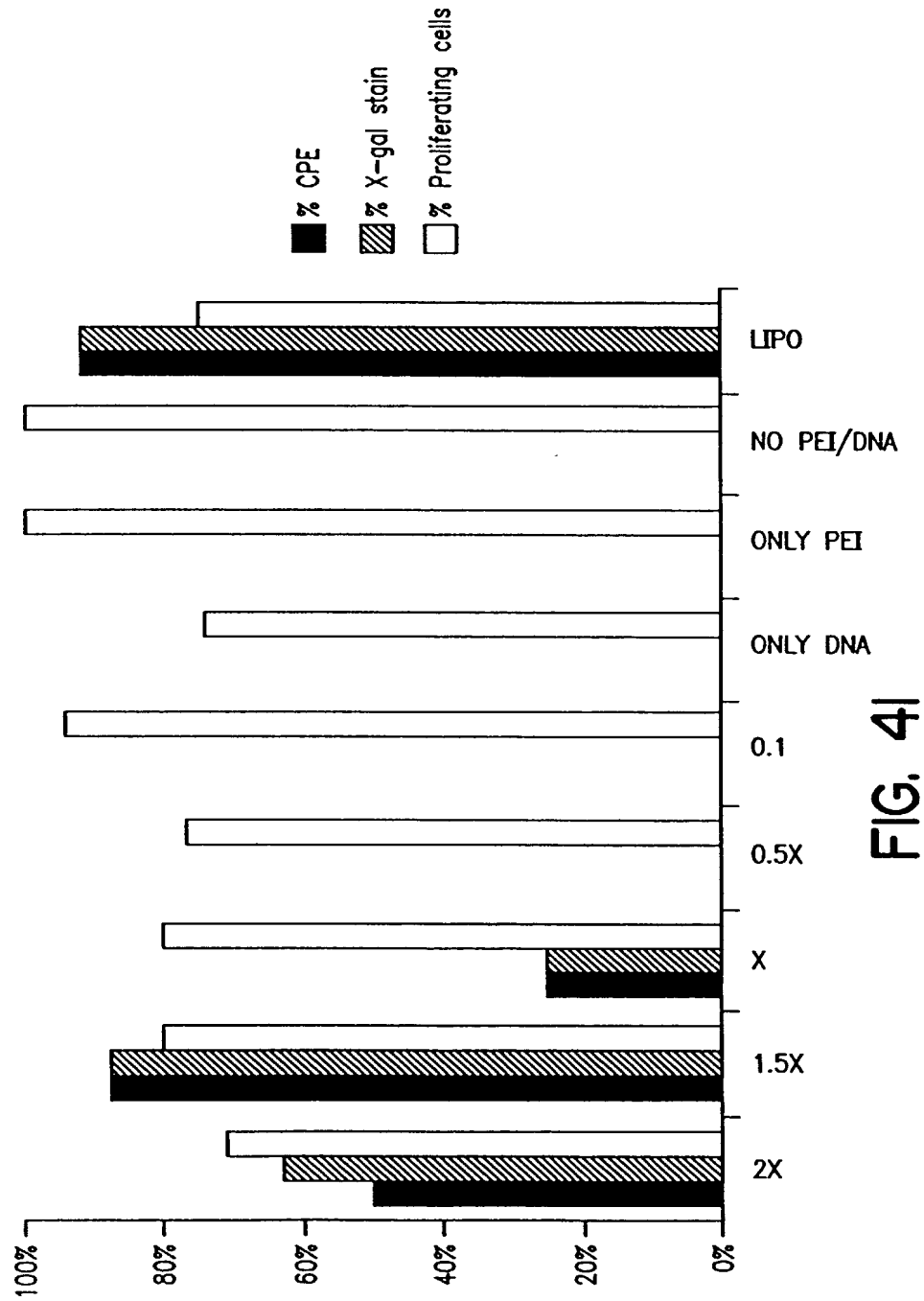


FIG. 40



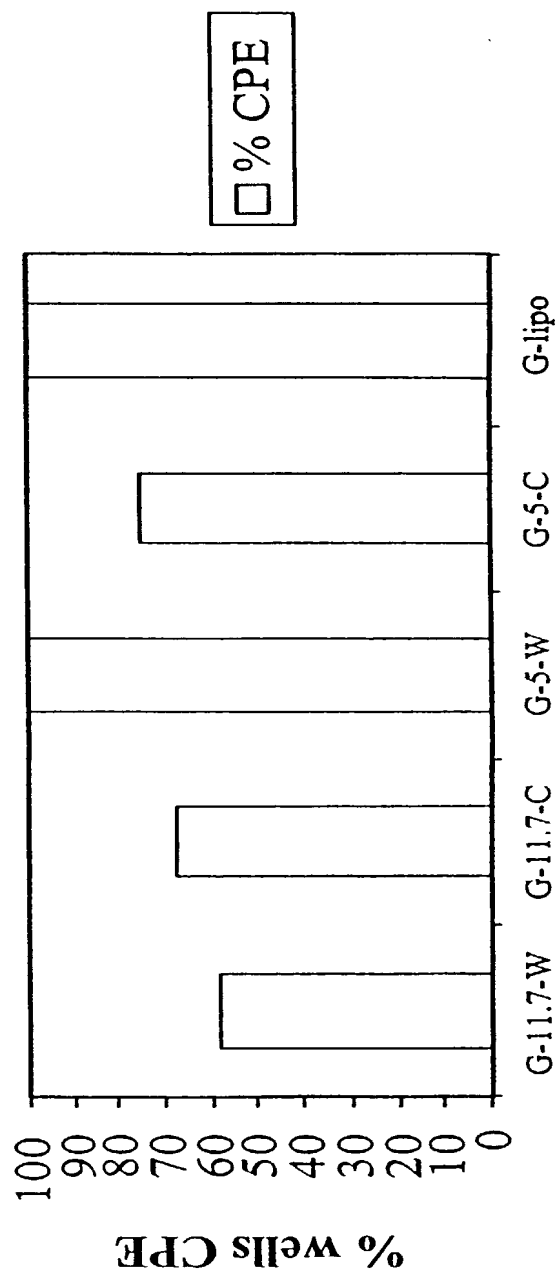


FIG. 42

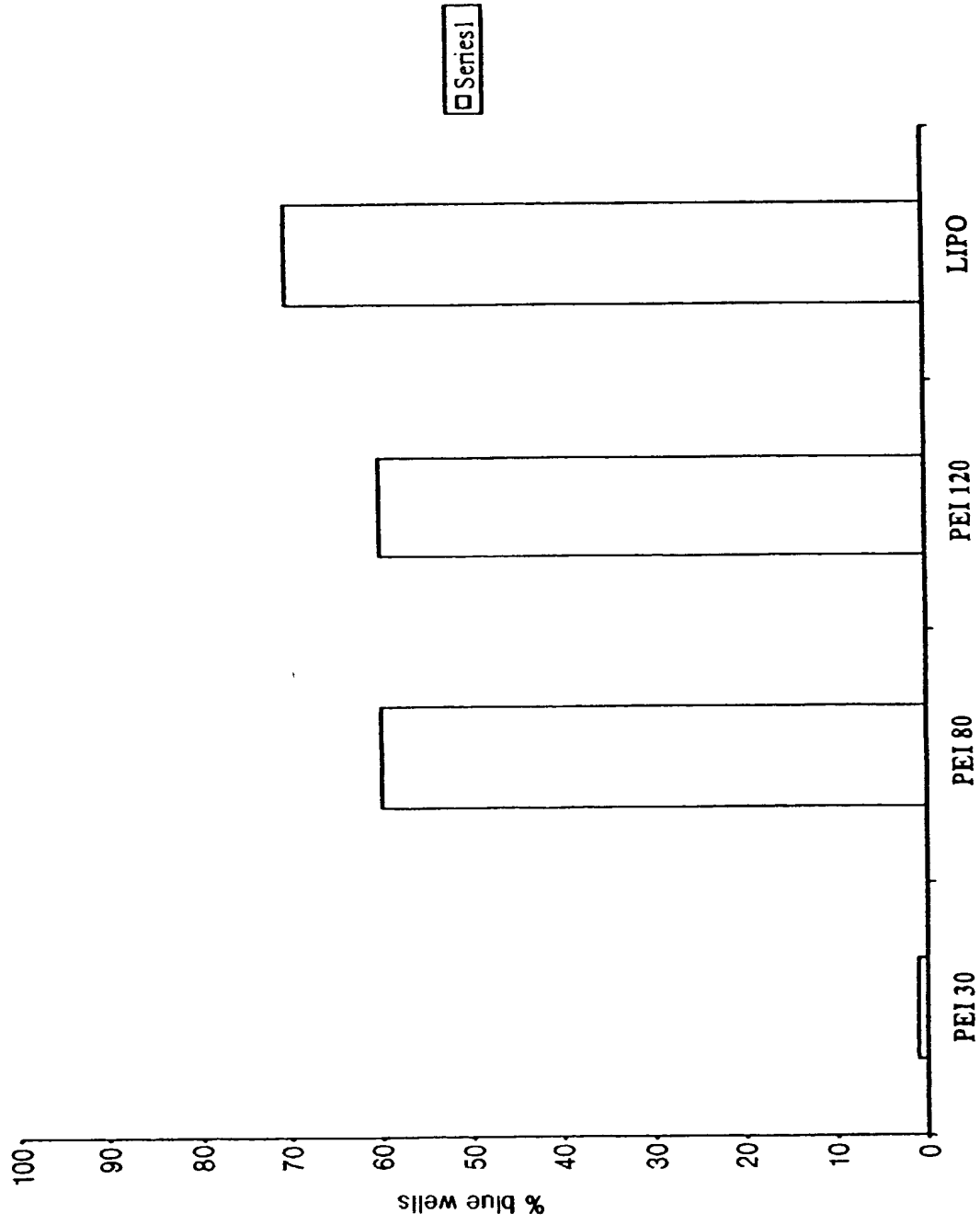


Figure 45

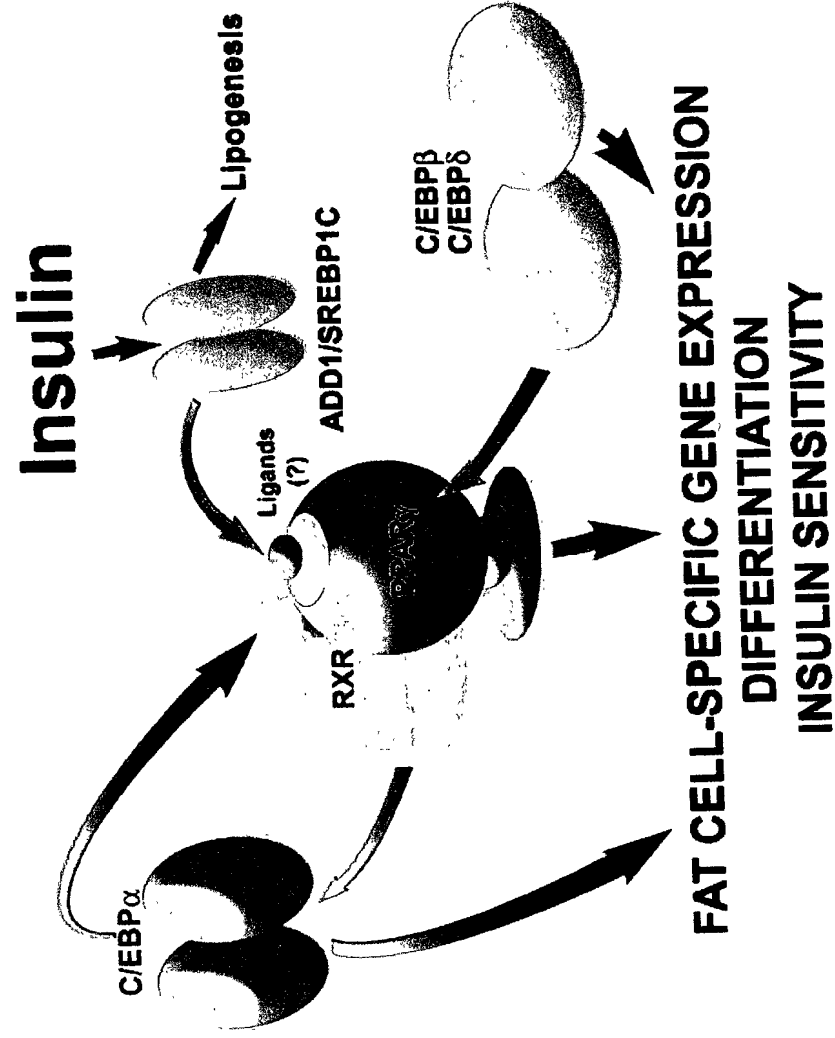


Figure 46

Transduction of hCAR⁻ cells with Ad5

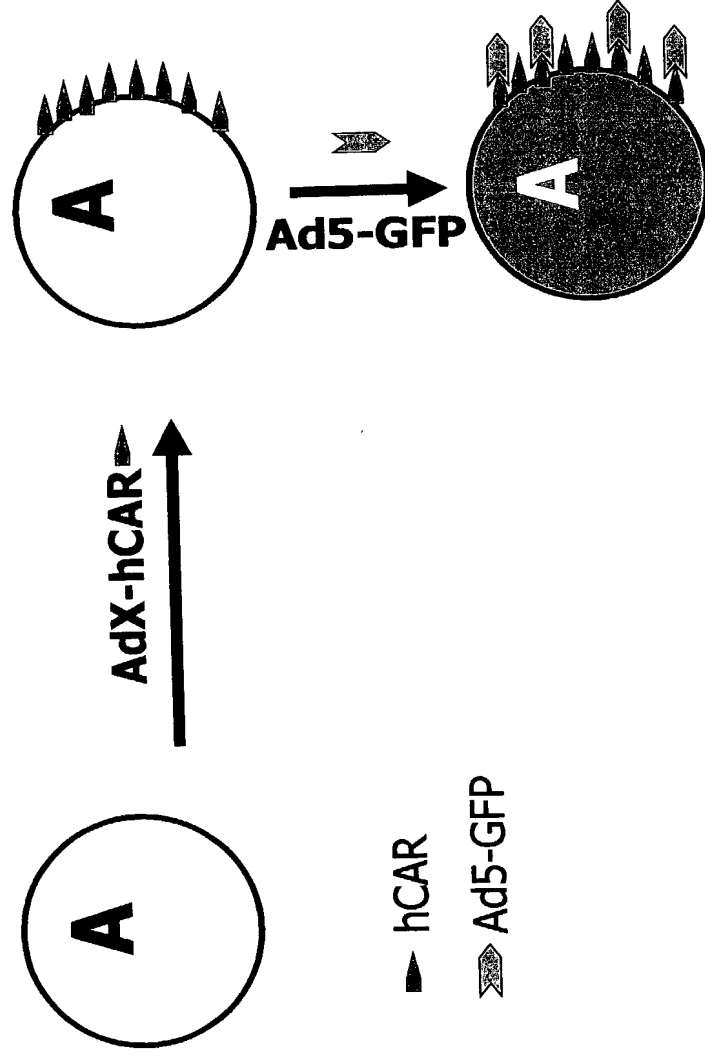
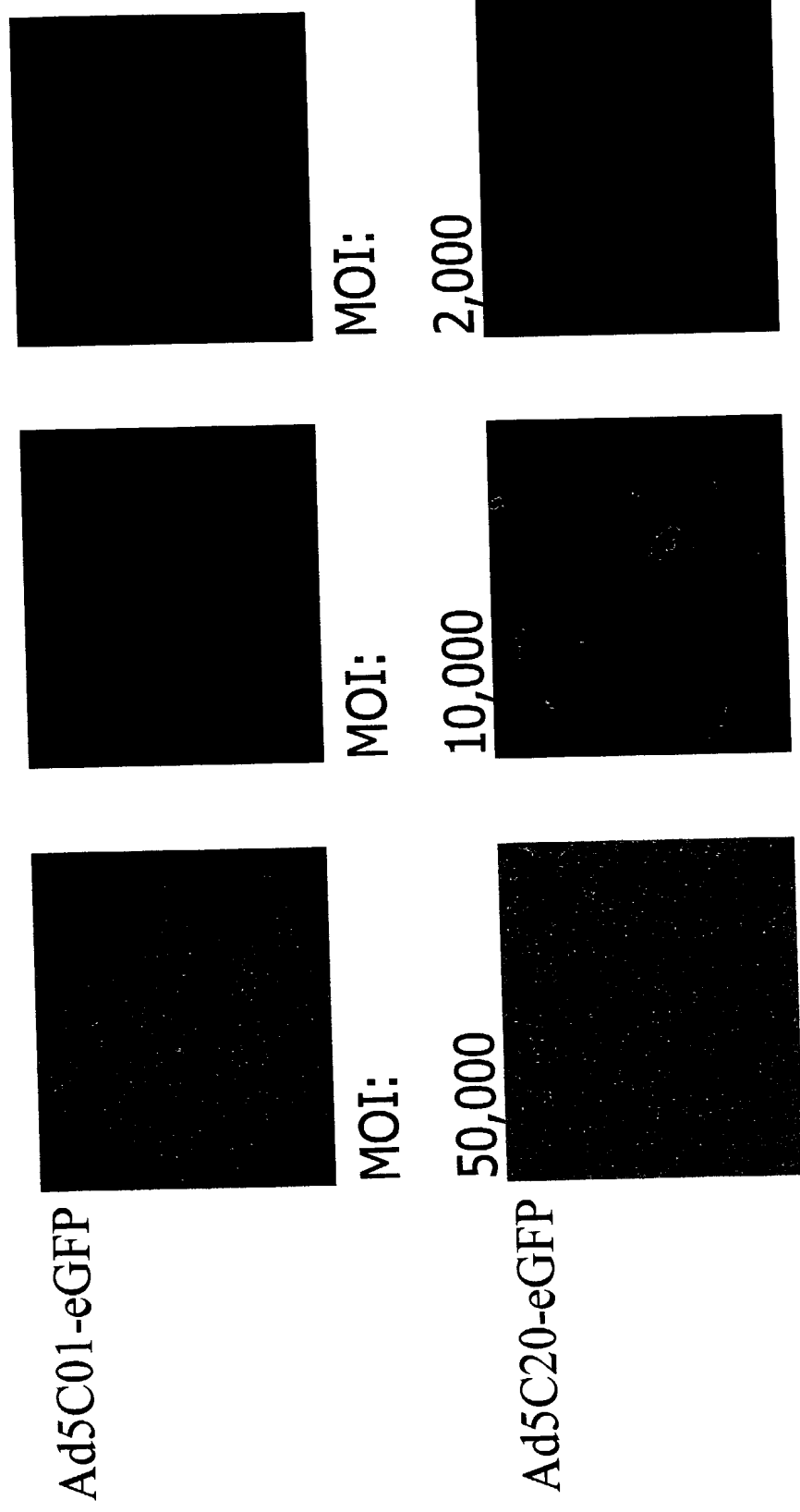
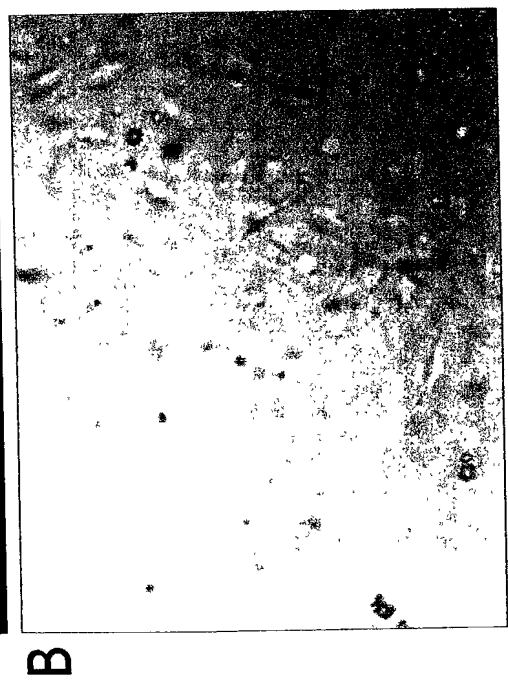
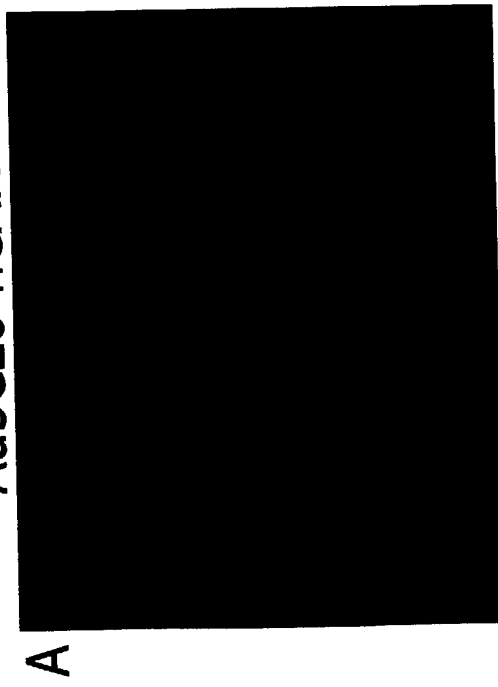


Figure 47

Infection of human primary pre-adipocytes using Ad5C01 and Ad5C20 fiber-modified viruses

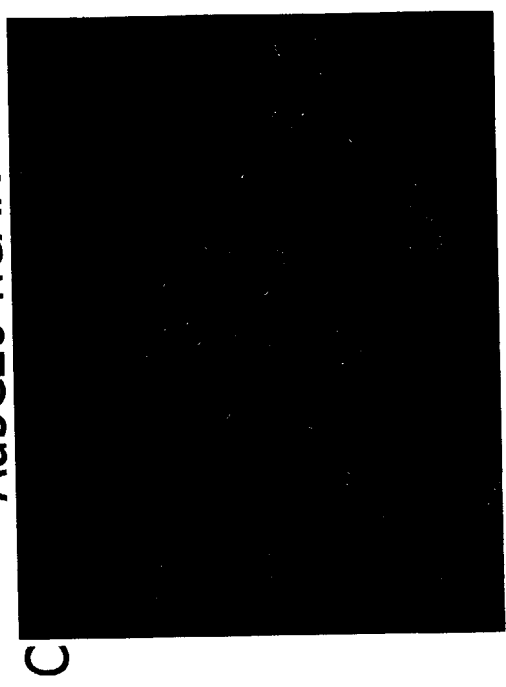


Ad5C01-Empty
+
Ad5C20-hCAR



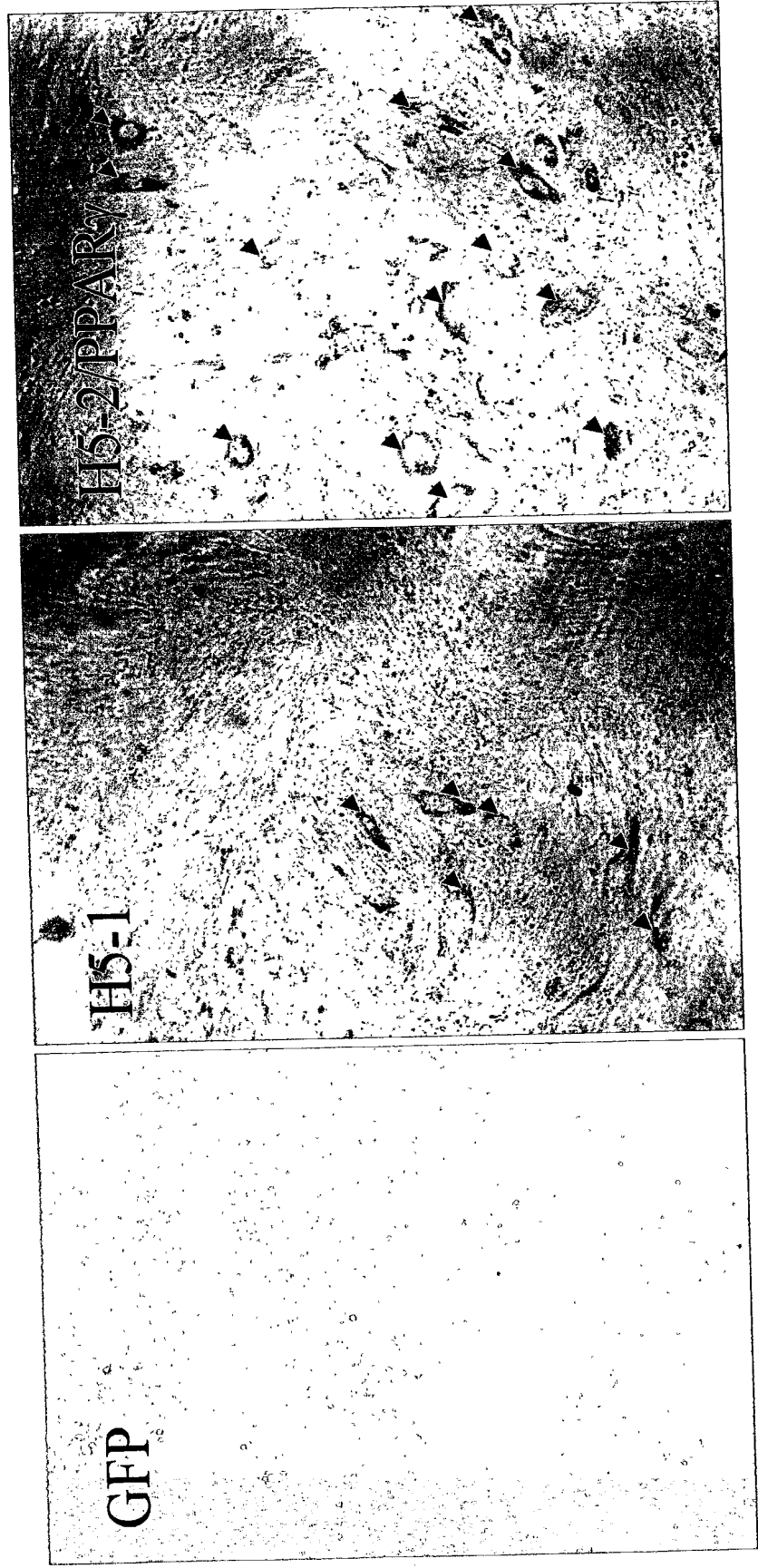
Ad5C01-PPAR γ
+
Ad5C20-hCAR

Figure 48



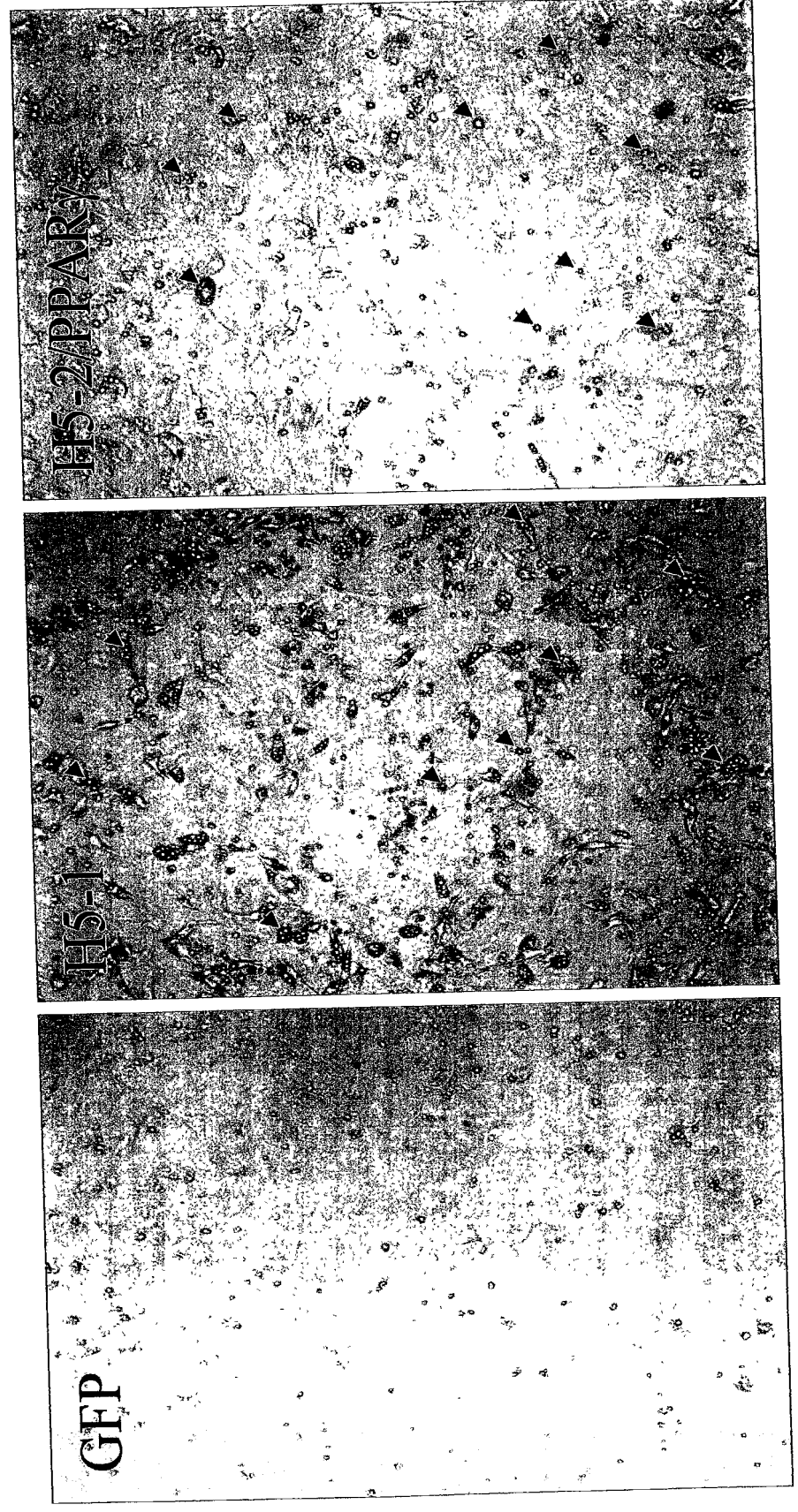
Adipocyte differentiation
Primary human mesenchymal stem cells

Figure 49



Adipocyte differentiation
Mouse mesenchymal stem cell line
C3H10T1/2

Figure 50



H5-24: adenovirally mediated expression
of CIDEB does not induce any cell death

Figure 51

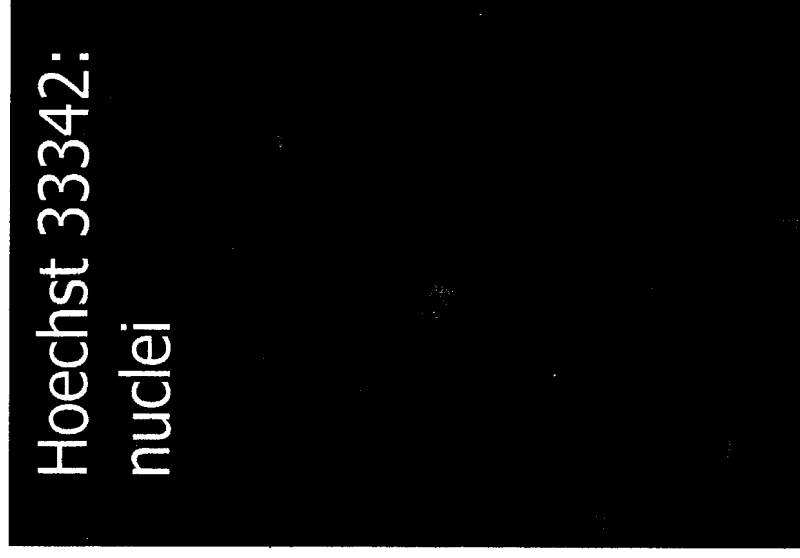
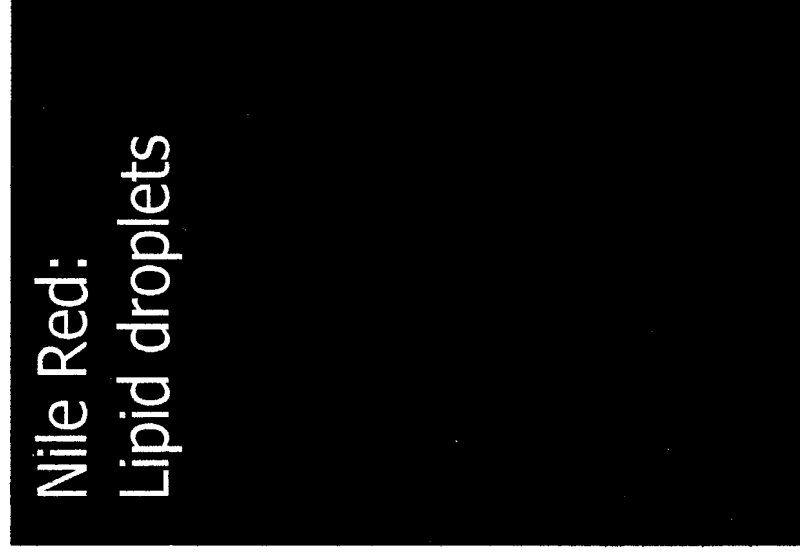
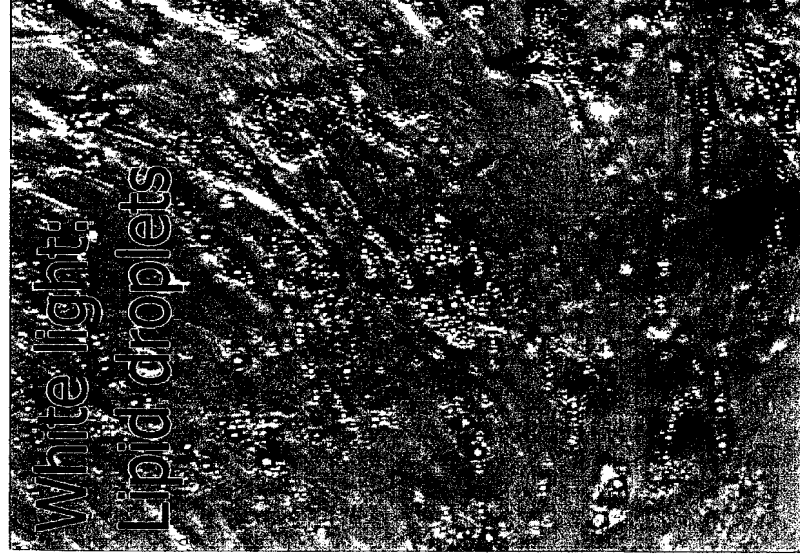


FIGURE 52

H5-1 DNA sequence (SEQ ID NO:12)

```

1  GCCCACGCGT  CCGGTTTTCT  ACTTTGCCAC  AGATTATCTT  GTACAGCCTT  TTATGGACCA
61  ATTAGCATTC  CATCAATTTT  ATATCTAGCA  TATTTGCGGT  TAGAATCCCA  TGGATGTTTC
121  TTCTTTGACT  ATAACAAAAT  CTGGGGAGGA  CAAAGGTGAT  TTTCTGTGT  CCACATCTAA
181  CAAAGTCAAG  ATTCCCGGCT  GGACTTTTGC  AGCTTCCTTC  CAAGTCTTCC  TGACCACCTT
241  GCACTATTGG  ACTTTGGAAG  GAGGTGCCTA  TAGAAAACGA  TTTTGAACAT  ACTTCATCGC
301  AGTGGACTGT  GTCCCTCGGT  GCAGAACTA  CCAGATTTGA  GGGACGAGGT  CAAGGAGATA
361  TGATAGGCCC  GGAAGTTGCT  GTGCCCCATC  AGCAGCTTGA  CGCGTGGTCA  CAGGACGATT
421  TCACTGACAC  TCGGAACCTC  CAGGACTACC  GTTACCAAGA  GGTTAGGTGA  AGTGGTTTAA
481  ACCAAACGGA  ACTCTTCATC  TTAAACTACA  CGTTGAAAAT  CAACCCAATA  ATTC'TGTATT
541  AACTGAATTC  TGAACCTTTC  AGGAGGTACT  GTGAGGAAGA  GCAGGCACCA  GCAGCAGAA'T
601  GGGGAATGGA  GAGGTGGGCA  GGGGTTCCAG  CTTCCCTTTG  ATTTTTTGCT  GCAGACTCAT
661  CCTTTTAAA  TGAGACTTGT  TTTCCCCTCT  CTTTGAGTCA  AGTCAAATAT  GTAGATTGCC
721  TTTGGCAATT  CTTCTTCTCA  AGCACTGACA  CTCATTACCG  TCTGTGATTG  CCATTTCTTC
781  CCAAGGCCAG  TCTGAACCTG  AGGTTGCTTT  ATCCTAAAAG  TTTTAACCTC  AGGTTCCAAA
841  TTCAGTAAAT  TTTGGAAACA  GTACAGCTAT  TTCTCATCAA  TTCTCTATCA  TGTTGAAGTC
901  AAATTTGGAT  TTTCCACCAA  ATTCTGAATT  TGTAGACATA  CTTGTACGCT  CACTTGCCCC
961  AGATGCCTCC  TCTGTCCTCA  TTCTTCTCTC  CCACACAAGC  AGTCTTTTTT  TACAGCCAGT
1021  AAGGCAGCTC  TGTCGTGGTA  GCAGATGGTC  CCATTATTCT  AGGGTCTTAC  TCTTTGTATG
1081  ATGAAAAGAA  TGTGTTATGA  ATCGGTGCTG  TCAGCCCTGC  TGTCAGACCT  TCTTCCACAG
1141  CAAATGAGAT  GTATGCCCAA  AGACGGTAGA  ATTAAAGAAG  AGTAAAATGG  CTGTTGAAGC
1201  AAAAAAAAAA  AAAAA

```

FIGURE 53

H5-24 DNA sequence (SEQ ID NO:14)

```

1  GTCGACCCAC GCGTCCGCGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61  CCGAAGGCCA GCACGATGGC GCTCACCAGC CGGCCACCC GCGCCCCGTG CCGCCCCGAG
121  CCCCAGCGGG CGCCCCGAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCCG
181  AAAGGAAGCA CGAAAGCGGT GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241  CAGCCAGACC GCCAGCAGCA GGCGGCCGGG CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301  GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361  GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCGCCTT GGCCCCAGCG
421  CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481  GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541  GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601  CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCAGCTC AGCAGTGTCT CGTTCCCTGG
661  GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGGAC ACAGAAAAAA AGTGGGGAAG
721  CTGGGGGACC CCTACAAGGA TCCTTGGCAG GAAAGCAGGG ATTGTGTCA TTTGAGGGTT
781  TCACTGTCTG TGAGAGTCTC AGCTTCCATG CAACTGTCCA TCACGGCTGC AACTGAAATC
841  AGAGCTGGGA CACAGCGCAC CAGAAGCTAA AGTCTTGATG CCATCAAAGG ACATCCCTGC
901  CCCATTCACT TCTCTGTCTC GTCCACTAAT CGGCAAAAGG AGAAAAGTGA GAGAAGATGA
961  CCTAAGTGTG ACTGCAGCAG GCAGCTCTGG AAAATGAAGC CAGAGCAGTG AGCCAGCCCC
1021  TCCTCCGACC AAGGAGGAAG GAAAGAGCAG CCCCAGCACA GGAGAGAACC ACCCAGCCCCA
1081  GAAGTTCCAG GGAAGGAAGT CTCCGGTCCA CCATGGAGTA CCTCTCAGCT CTGAACCCCA
1141  GTGACTTACT CAGGTCAGTA TCTAATATAA GCTCGGAGTT TGGACGGAGG GTCTGGACCT
1201  CAGCTCCACC ACCCCAGCGA CCTTTCCGTG TCTGTGATCA CAAGCGGACC ATCCGGAAAG
1261  GCCTGACAGC TGCCACCCGC CAGGAGCTGC TAGCCAAAGC ATTGGAGACC CTAAGTCTGA
1321  ATGGAGTGCT AACCTGGTG CTAGAGGAGG ATGGAAGTGC AGTGGACAGT GAGGACTTCT
1381  TCCAGCTGCT GGAGGATGAC ACGTGCCTGA TGGTGTGCA GTCTGGTCAG AGCTGGAGCC
1441  CTACAAGGAG TGGAGTGCTG TCATATGGCC TGGGACGGGA GAGGCCAAG CACAGCAAGG
1501  ACATCGCCCC ATTACCTTT GACGTGTACA AGCAAAACCC TCGAGACCTC TTTGGCAGCC
1561  TGAATGTCAA AGCCACATTC TACGGGCTCT ACTCTATGAG TTGTGACTTT CAAGGACTTG
1621  GCCCAAAGAA AGTACTCAGG GAGCTCCTTC GTTGGACCTC CAACTGCTG CAAGGCCTGG
1681  GCCATATGTT GCTGGGAATT TCCTCCACCC TTCGTCATGC AGTGGAGGGG GCTGAGCAGT
1741  GGCAGCAGAA GGGCCGCTC CATTCCTACT AAGGGGCTCT GAGCTTCTGC CCCAGAATC
1801  ATTCCAACCG ACCCACTGCA AAGACTATGA CAGCATCAAA TTTCAGGACC TGCAGACAGT
1861  AAGGCTAGA TAACCCACCC AATTTCCCCA CTGTCCTCTG ATCCCCTCGT GACAGAACCT
1921  TTCAGCATAA CGCCTCACAT CCCAAGTCTA TACCTTACC TGAAGAATGC TGTCTTTTCC
1981  TAGCCACCTT TCTAGCCTCC CACTTGCCCT GAAAGGCCAA GATCAAGATG TCCCCAGGC
2041  ATCTTGATCC CAGCCTGACT GCTGCTACAT CTAATCCCCT ACCAATGCCT CCTGTCCCTA
2101  AACTCCCCAG CATACTGATG ACAGCCCTCT CTGACTTTAC CTTGAGATCT GTCTTCATAC
2161  CCTTCCCCTC AAATAACAA AACATTTTCC AATAAAAATA TCAAATATTT AAAAAAAAAA
2221  AAAAAAAGGG CGGCCGCG

```

FIGURE 54

H5-24 ORF4 Amino Acid sequence (SEQ ID NO: 71)

MEYLSALNPSDLLRSVSNISSEFGRRVWTSAPPPQRPFRVCDHKRTIRKGLTAAT
RQELLAKALETLLLNGVLTTLVLEEDGTAVDSEDDFFQLLEDDTCLMVLQSGQSW
PTRSGVLSYGLGRERP KHSKDIARFTFDVYKQNPRDLFGSLNVKATFYGLYSMS
CDFQGLGPKKVLRELLRWTSTLLQGLGHMLLGISSTLRHAVEGAEQWQKGR
LSY

FIGURE 55

H5-24 Segment 1 of BLTR2 DNA sequence (SEQ ID NO: 15)

```
18 CGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61 CCGAAGGCAA GCACGATGGC GCTCACCAGC CGGCCCACCC GCGCCCCGTG CCGCCCGGAG
121 CCCCAGCGGG CGCCCCGCAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCG
181 AAAGGAAGCA CGAAAGCGGT 200
```


FIGURE 56

H5-24 Segment 2 DNA sequence (SEQ ID NO: 16)

```
198 GGT GGC GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241 CAGCCAGACC GCCAGCAGCA GGCGGCCGGG CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCAGCGG
421 CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541 GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601 CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCCAGCTC AGCAGTGTCT CGTTCCCTGG
661 GGGACGGTAG CAGACCGACA TCC'TTCTGGG CCTACAGG 698
```

FIGURE 57

DNA Sequence Comparison of H5-24 Segment 1 (SEQ ID NO: 15) with BLTR2

Antisense DNA sequence

| | | | |
|--------------|------|--|------|
| SEQ ID NO:15 | 18 | cgcctgcagaaggttgactgcgtggtagggggcccagagcaagccaaggaagcacgat | 77 |
| | | | |
| BLTR2 | 2455 | cgcctgcagaaggttgactgcgtggtagggggcccagagcaagccaaggaagcacgat | 2396 |
| SEQ ID NO:15 | 78 | ggcgctcaccagccggccccacccgcgccccgtgccgcccggagccccagcggcgccccg | 137 |
| | | | |
| BLTR2 | 2395 | ggcgctcaccagccggccccacccgcgccccgtgccgcccggagccccagcggcgccccg | 2336 |
| SEQ ID NO:15 | 138 | cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaaggaagcacgaaagc | 197 |
| | | | |
| BLTR2 | 2335 | cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaaggaagcacgaaagc | 2276 |
| SEQ ID NO:15 | 198 | ggt | 200 |
| | | | |
| BLTR2 | 2275 | ggt | 2273 |

FIGURE 58

DNA Sequence Comparison of H5-24 Segment 2 (SEQ ID NO: 16) with BLTR2

Antisense DNA sequence

| | | | |
|--------------|------|--|------|
| SEQ ID NO:16 | 198 | ggtggcggtagacggcgccgggacggcgagcaacagggcgccagccagaccgccagca | 257 |
| | | | |
| BLTR2 | 2195 | ggtggcggtagacggcgccgggacggcgagcaacagggcgccagccagaccgccagca | 2136 |
| SEQ ID NO:16 | 258 | gcaggcgggcgccagggcgccggctgcgagccgagggcgccaggaagggcggtgactg | 317 |
| | | | |
| BLTR2 | 2135 | gcaggcgggcgccagggcgccggctgcgagccgagggcgccaggaagggcggtgactg | 2076 |
| SEQ ID NO:16 | 318 | cgaggcagcgctgcaggctgagcaggccggtgagcagcacgctggcgctacatgctgagcg | 377 |
| | | | |
| BLTR2 | 2075 | cgaggcagcgctgcaggctgagcaggccggtgagcagcacgctggcgctacatgctgagcg | 2016 |
| SEQ ID NO:16 | 378 | cgcacacgtagtacaccgccttgagcccgccctggcccagcgccagggcctgcccgggtca | 437 |
| | | | |
| BLTR2 | 2015 | cgcacacgtagtacaccgccttgagcccgccctggcccagcgccagggcctgcccgggtca | 1956 |
| SEQ ID NO:16 | 438 | ggaaggccacaaagagcggtgagcagcagcaccgcgcctcgccagcgccaggtgca | 497 |
| | | | |
| BLTR2 | 1955 | ggaaggccacaaagagcggtgagcagcagcaccgcgcctcgccagcgccaggtgca | 1896 |
| SEQ ID NO:16 | 498 | gcacaagcgtggccgccagcggtcgcccccggtgcaggcgccagcccgccaagctccaca | 557 |
| | | | |
| BLTR2 | 1895 | gcacaagcgtggccgccagcggtcgcccccggtgcaggcgccagcccgccaagctccaca | 1836 |
| SEQ ID NO:16 | 558 | ccacgaagccggttgccaggcagccccagcagcgcccgccagcagcaggaaggctgtgcctg | 617 |
| | | | |
| BLTR2 | 1835 | ccacgaagccggttgccaggcagccccagcagcgcccgccagcagcaggaaggctgtgcctg | 1776 |
| SEQ ID NO:16 | 618 | tggcccgcggaagtcttcagctcagcagtgctctcgttccctgggggacggtagcagaccg | 677 |
| | | | |
| BLTR2 | 1775 | tggcccgcggaagtcttcagctcagcagtgctctcgttccctgggggacggtagcagaccg | 1716 |
| SEQ ID NO:16 | 678 | acatccttctgggcctacagg | 698 |
| | | | |
| BLTR2 | 1715 | acatccttctgggcctacagg | 1695 |